CHARACTERIZATION OF HOST RESPONSE TO *EIMERIA TENELLA* INFECTIONS IN CHICKENS

By

T. K. JIT SINGH MUKKUR

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INTRODUCTION

Coccidiosis is one of the most prevalent parasitic diseases of poultry and as such has been the object of wide study and experimentation for the past forty years. This disease is caused by microscopic parasites belonging to the Phylum Protozoa, Class Sporozoa, Order Coccidia, and Genus *Eimeria*. Nine species of *Eimeria* have been described from the chicken (*Gallus domesticus*) all of which invade the epithelial cells of the intestinal tract producing the disease which is characterized by severe hemorrhage, followed by death.

Coccidiosis causes a great economic loss to the poultry industry throughout the world. A total loss of $34,854,000 was estimated to the poultry industry from 1951 to 1960 by the United States Department of Agriculture (1965). Of this sum, $15,123,000 were attributed to mortality and $19,731,000 to morbidity. At the present time, coccidiosis is controlled by the use of coccidiostatic drugs, the annual cost of which was estimated at $25,000,000 in the United States (Reid, 1963). However, there are certain drawbacks to the use of drugs; for example, interference with immunity (Davies and Kendall, 1955; Reid, 1960), side effects of the drugs on important factors such as fertility (Joyner, 1965),

and the production of drug-fast strains (McLoughlin and Gardiner, 1961a, 1961b, 1962; Pellerdy, 1962; Gardiner and McLoughlin, 1963; Vegh, 1963). Moreover, no single drug is known that would offer equal protection against all species because no drug is considered entirely suitable for administration throughout the life of the bird. Much depends on the degree of infection in the environment and the effectiveness of the drug used (Joyner, 1964). Outbreaks of coccidiosis may still occur in birds which appear to have been adequately protected.

Under natural conditions chickens often develop a varying degree of resistance due to exposure to mild infections of Eimeria species, notably with E. tenella. The development of acquired immunity by this means was first reported by Johnson (1927) and confirmed by numerous subsequent workers, but immunity to reinfection with E. tenella is by no means absolute. As ordinarily understood, an immune bird is one that is resistant to the clinical effects of the disease. An absolute resistance to reinfection appears to develop in some adult birds which have been exposed to infection on more than one occasion and there is no apparent development of the parasite. In general, however, only a condition of relative immunity exists (Levine, 1963).

A great deal of work has been reported by numerous workers in search for a satisfactory method of conferring solid immunity to chickens by administration of a known number of altered or unaltered oocysts of E. tenella

(Johnson, 1927; Tyzzer, 1929; Jankiewicz and Scofield, 1934; Waxler, 1941b; Farr, 1943; Rose and Long, 1962; Pierce *et al.*, 1962). The nature of this immunity is still problematic, and no one basis for resistance to *E. tenella* is universally accepted. In general, there are two schools of thought concerning the nature of immunity to infections with *E. tenella*. The theory that cellular defense mechanisms are operative in some way received the support of Tyzzer (1929), Ripsom *et al.* (1949), and Pierce and Long (1965). Although numerous attempts have been made to incriminate the humoral antibody as responsible for resistance (Burns and Challey, 1965; Herlich, 1961; Long *et al.* 1963), it is not definitely known whether the humoral antibody is produced in the birds after infection with *E. tenella*, although scattered conflicting reports are available (Pierce *et al.*, 1962; McDermott and Stauber, 1954).

The purpose of the present investigation was to characterize the host response of chickens infected or superinfected with *E. tenella* oocysts, by making the following physiological and immunological measurements.

1. Hematological observations including determinations of packed cell volume, hemoglobin, and white blood cell response.

2. Serum analysis including the determination of total serum protein and various fractions such as albumin and alpha-1, alpha-2, beta, and gamma globulins.

3. Protection studies including the challenge of birds infected or immunized with a single dose or two or three graded doses of _E. tenella_ oocysts. _E. tenella_ was selected to be used for this investigation since it can be easily obtained in pure culture without contamination with other coccidial species due to its marked organ specificity for the ceca of the birds (Herrick, 1936). It is hoped that this investigation will contribute to a greater understanding of the host-parasite relationship in coccidial infections.

LITERATURE REVIEW

Nine pathogenic species belonging to genus *Eimeria* have been described from the alimentary tract of chickens: *Eimeria acervulina*, *E. brunetti*, *E. hagani*, *E. maxima*, *E. mitis*, *E. mivati*, *E. necatrix*, *E. praecox*, and *E. tenella* (Biester and Schwarte, 1965). All of these species parasitize specific portions of the intestinal tract except *E. mivati*, which may be found in several regions (Edgar and Seibold, 1964). These species can be differentiated from one another by the application of both morphological and biological characters. Of the morphological criteria, the structure of the oocyst is usually used to identify the species, at least within a given host (Levine, 1961).

One of the major biological differentiations for *Eimeria* species is the location of the endogenous stages in specific regions of the intestinal tract of the host, since a marked regional specificity has been reported for the coccidia species (Tyzzer, 1929; Tyzzer *et al.*, 1932; Herrick, 1936). A second biological criterion is that of immunity. Infection of a chicken with a given coccidia species results in immunity against that species but not against others even within the same host. Therefore, cross immunity tests can be used to differentiate the various coccidial species (Tyzzer, 1929).

Life History and Morphology of *Eimeria tenella*

Eimeria tenella (Railliet and Lucet, 1891) is the most common and the most pathogenic of all the coccidia of chickens and is the only one that is regularly associated with outbreaks of acute disease (cecal coccidiosis) with a high rate of mortality (Davies *et al.*, 1963).

Tyzzer (1929) published a detailed description of the morphology and life history of *E. tenella* which has been confirmed in all essential details by subsequent investigators.

The unsporulated oocysts of *E. tenella*, which are passed in the feces of infected birds, are ovoidal in shape with no apparent micropyle. There is a considerable variation in the size of the oocysts, from 19-26 microns long and 16.5-22.8 microns wide (Tyzzer, 1929). Within the oocyst, the cytoplasmic mass appears slightly irregular and is separated from the wall by a relatively large clear space. Within the oocyst wall, at the anterior end, a small bright refractile granule is present. Sporulation occurs in approximately 48 hours, when the oocyst is kept at room temperature with sufficient oxygen and moisture. The sporulated oocyst contains four sporocysts or spores, each containing two sporozoites. Each spore is bluntly ovoid in shape and measures about seven microns in width and 11 microns in length. At the smaller end, a small globular plug fills an opening in the sporewall

and projects slightly outward. The sporozoites are small, sausage-shaped forms, with a globular mass of hyaline material near one end and a space devoid of any granules which is thought to be the nucleus. There is no residual mass within either the oocyst or the sporocyst.

When ingested by a chicken, sporozoites are released from the oocyst, although the factors which cause excystation have not been definitely established. It was suggested by Itagaki and Tsubokura (1958) that pancreatic juice was not responsible for excystation. Levine (1942), on the other hand, failed to obtain infection of chickens with _E. tenella_ or other species of coccidia when the pancreatic ducts were ligated. Ikeda (1960) incriminated pancreatic juice, in particular trypsin, as responsible for the excystation of _E. tenella_ and Goodrich (1944) successfully liberated sporozoites from sporulated oocysts by treatment with trypsin _in vitro_. This finding was later confirmed by Farr and Doran (1961).

Once liberated from the oocyst, the sporozoites invade the cecal epithelium, penetrating the basement membrane of individual epithelial cells to enter the tunica propria through which they pass, either free or within macrophages, and finally invade the epithelial cells lining the gland of Lieberkühn where they are found below the host cell nucleus (Challey and Burns, 1959; Pattillo, 1959). Once in a glandular epithelial cell, the sporozoite rounds up and becomes a first generation schizont, within 24 to 48 hours. The first generation schizonts, located

at the bottom of the crypts of the cecal glands, measure about 24 microns in diameter. The growth of the schizont results in an increase in the size of the epithelial cell which bulges into the lumen of the cecum and releases merozoites into the cecal lumen two to three days after infection. Each schizont forms about 900 merozoites (Tyzzer, 1929) (each of which is two-four microns in length and one-1.5 microns in width) by a process of asexual multiple fission known as schizogony. Each first generation merozoite enters a new host cell, rounds up, increases in size and actively migrates into subepithelial layers of tissue to form the second generation schizont. Growth of the second generation schizont is rapid and, 24 hours later, mature schizonts measuring about 25-54 microns in length and 22.5-40.4 microns in width (Tyzzer, 1929) containing numerous second generation merozoites are developed. Subsequently, the merozoites are released into the cecum due to the destruction of the overlying epithelial cells. The maturation of vast numbers of parasites in the cecal epithelium and the resultant cellular destruction results in hemorrhage which commences at about the 96th hour. The second generation merozoites are considerably larger than the first, averaging about 16 microns in length, two microns in width, and 200-350 in number, many of which enter new host cells and begin the sexual phase of the life cycle known as gametogony. The majority of these merozoites become macrogametes which are as large as the oocyst, while a smaller number becomes micro-

gametocytes each of which may vary from 5.5-18.8 microns in length. Both the macrogametocytes and the microgametocytes lie below the host cell nuclei. Within each microgamete are formed a large number of tiny biflagellate microgametes, one of which after liberation, fertilizes each macrogamete. The resultant zygote lays down a wall around itself in the following manner: the eosinophilic plastic granules of the cytoplasm of the macrogamete, composed of mucoprotein, pass to the periphery, flatten out and coalesce to form the oocyst wall after fertilization, which marks the transition of a fertilized macrogamete into an oocyst (Kheisin, 1958). The outer layer of the oocyst wall is a quinone-tanned protein and the inner layer is a lipid coat firmly associated with a protein lamella (Monné and Hönig, 1954). Ultimately the oocysts break out of their host cells, enter the intestinal lumen and are passed out in the feces. The entire period from the time of infection of the birds with sporulated oocysts to the appearance of the first unsporulated oocysts in the feces lasts approximately seven days and is known as the prepatent period.

Pathogenesis of Cecal Coccidiosis

Factors affecting pathogenicity of _E. tenella_ include the size of the infecting dose of oocysts, the number of host cells destroyed per infecting oocyst, the degree of reinfection and the degree of immunity in the host. The final effect depends on the interplay of all

these factors and may range from an imperceptible reaction to death (Gardiner, 1955).

Age is especially an important factor since cecal coccidiosis is primarily a disease of young birds. The range of age of susceptibility is from two weeks to 15 months, chickens being most susceptible at eight weeks of age (Herrick, 1936; Herrick *et al.*, 1936; Gardiner, 1955). Those birds which recover from infection with *E. tenella* become immune to reinfection with this organism; however, it is not an absolute immunity. Older birds may be continuously reinfected, becoming carriers and disseminating *E. tenella* infections to other chickens. Under conditions of stress, the acquired immunity of older birds may break down, causing symptoms of the disease to reappear (Levine, 1963).

The prepatent period in *E. tenella* infection is seven days, but the patent period varies with individual infections. Fish (1931) reported that oocysts were not present in the droppings of the infected birds after 17 days, although Tyzzer *et al.* (1932) recorded oocyst passage for as long as 19 days post-infection. The greatest numbers of oocysts are discharged in a very short time (Tyzzer *et al.*, 1932), the few remaining being trapped either in the tissues or in the cecal contents and irregularly released. Under natural conditions, birds are usually infected repeatedly and thus may pass oocysts for much longer periods of time. For example, Levine (1940) observed oocysts of *E. tenella* in the fecal droppings

of nine out of 30 birds which did not show any symptoms of infection.

The severity of cecal coccidiosis depends on the number of sporulated oocysts that the bird receives (Johnson, 1927). For instance Jankiewicz and Scofield (1934) reported that a dosage of up to 150 sporulated oocysts produced neither symptoms nor mortality; 150 to 500 oocysts produced slight hemorrhage and no mortality; 1,000 to 3,000 oocysts, a fairly heavy degree of hemorrhage and moderate mortality; and over 5,000 oocysts produced severe hemorrhage and high mortality. The disease symptoms in cecal coccidiosis are closely related to the course of infection. On the fourth day after infection birds appear listless, due to the growth of second generation schizonts and resultant hemorrhage. The passage of large quantities of blood in the droppings on the fifth and sixth day after infection is due to the breaking out of the second generation merozoites and widespread sloughing of the cecal mucosa. The infected birds consume less feed but often consume two or three times more water than the uninfected birds. Approximately 90 per cent of the mortality occurs within the first week following infection, and if the birds do not die within this time, recovery follows.

Diagnosis of cecal coccidiosis is based on the appearance of blood-filled ceca at necropsy. No other disease condition in poultry resembles cecal coccidiosis to any appreciable extent (Levine, 1961).

Pathology of Cecal Coccidiosis

The lesions associated with E. tenella infection occur primarily in the ceca and have been described by Tyzzer (1929), Tyzzer et al. (1932), and Mayhew (1937). The dilated part of the cecum is primarily involved. If birds are killed on the fourth day after infection, hemorrhage is found throughout the cecal mucosa. The cecum is usually filled with unclotted or partially clotted blood on the fifth day after infection, at which time the feathers and the skin about the vent may be stained with blood. By the sixth day post-infection, the cecum is grossly dilated with clotted blood. Cecal cores, composed of fibrin and cecal contents, may be found by the seventh day. These are tightly adherent to the mucosa, but become detached and free later within the lumen. Occasionally a core or blood clot is passed intact in the droppings of an infected bird. After infection, the cecum assumes its usual gross appearance, although it may become slightly larger and thickened. The greatest damage is apparently caused by the enlargement of the second generation schizonts in the lamina propria of the cecal wall, and the extensive sloughing of the cecal epithelium on the fifth day after infection is associated with the release of the second generation merozoites (Morgan and Hawkins, 1955).

In light infections, the regeneration of the epithelium is complete but in more severe cases, the recovery

is associated with slow and often incomplete regeneration of the mucosa.

Natt and Herrick (1955) observed that the erythrocyte count and hematocrit decreased to about 50 per cent of normal on the fifth and sixth day following an infection with 50,000 E. tenella oocysts and eight days were required for these values to return to normal. Natt (1959) observed lymphocytopenia and heterophilia on the fifth day and an eosinophilia on the tenth day following an infection with E. tenella. No significant changes were observed in the monocytes and basophil numbers during the course of infection. A marked leukocytosis began on the seventh day post-infection and persisted through the recovery phase of the disease.

An increase in blood sugar was observed by Pratt (1940) during the acute stages of the disease accompanied by a decrease in the muscle glycogen. Waxler (1941a) reported an increase in blood chlorides on the sixth and seventh day following infection with E. tenella but the rise in blood sugar was apparent one day earlier, i.e., five days post-infection. The chloride content of the muscle showed a downward trend and could account in part for the rise in blood chlorides. Daugherty and Herrick (1952) reported the production of a substance in the cecum during the acute stages of infection which reduced the capacity of chicken brain to utilize glucose but not hexose diphosphate. This led them to suggest that, in

part, the symptoms of cecal coccidiosis were due to interference with normal phosphorylative carbohydrate utilization.

An increase in adrenal ascorbic acid and adrenal corticosterone concentrations was observed in chicks infected with E. tenella (Challey, 1962). This change was reported to take place during the acute hemorrhagic phase of the infection.

Immunity to Cecal Coccidiosis

Chickens often show a high degree of resistance to infection with E. tenella under natural conditions, which could be due to picking up small amounts of infective material resulting in the development of acquired immunity according to Johnson (1927), who orally inoculated chickens with 2,000 sporulated oocysts of E. tenella daily for 15 days and was successful in inducing an immunity which lasted for six and one-half months. Farr (1943) used 1,000 oocysts on the same schedule and recorded the development of a strong immunity which lasted at least 14 months as judged by the lack of hemorrhage or mortality on challenge by inoculation with a large number of oocysts. Similar results were obtained when a total of 15,000 oocysts were administered in three doses of 1,000, 5,000, and 9,000 oocysts at five-day intervals. Rose and Long (1962) reported that if chickens were orally inoculated with 500 E. tenella oocysts followed by 5,000 on the seventh day and 50,000 on the 14th day and challenged 14 days later

with 100,000 sporulated oocysts by the same route, they were completely resistant. In such birds, when ten million oocysts were administered 21 days after the last of the graded doses, no detectable first generation schizogony developed.

Horton-Smith et al. (1963), while investigating the fate of invasive stages of E. tenella in the immune chicken, observed that sporozoites readily invaded the cecal epithelium of birds resistant to E. tenella infection but did not develop further and subsequently were not detected in tissue sections 72 hours after infection. Similarly, second generation merozoites, inoculated directly into the ceca of these resistant birds invaded the cecal tissue but did not grow further and were undetectable in the tissue sections 30 hours after infection. The sporozoites removed from the cecal lumen of these immune birds would produce infections in susceptible chickens equivalent to those produced by a similar number of sporozoites from the ceca of non-immune birds. On the other hand, Leathem and Burns (1967) reported that sporozoites removed from the cecal mucosa of immune chickens failed to initiate infections on inoculation into susceptible birds, whereas the isolates from non-immune birds readily induced the typical cecal coccidiosis infection.

Jankiewicz and Scofield (1934) reported that the oral inoculation of killed E. tenella oocysts failed to induce immunity. However, by approaching the problem from the standpoint of preventing the clinical symptoms

of cecal coccidiosis, these workers reported that some degree of resistance could be induced by use of sporulated oocysts which had been heated at 48° C for 20 minutes prior to inoculation. Waxler (1941b) reported the successful use of x-ray attenuated *E. tenella* oocysts for inducing immunity in birds against cecal coccidiosis. Uricchio (1953) was unable to produce an appreciable resistance in birds that were fed oocysts altered by means of ultrasonics, radium, or heat at 60° C.

All attempts to induce passive immunity by the use of hyperimmune serum have been unfruitful (Pierce *et al.*, 1963). Similarly, it has not been possible to transfer resistance to *E. tenella* infection from domestic hens to their progeny (Long and Rose, 1962). Likewise, the inoculation of peripheral white blood cells or spleen cells from an immune to a non-immune chicken did not effect the course of development of the second generation merozoites of *E. tenella* administered *per rectum*, in that the oocyst production in both groups was not significantly different (Horton-Smith and Long, 1963).

Horton-Smith *et al.* (1961), using chickens with one cecum ligated, infected them with *E. tenella* by oral inoculation and followed with a challenge 21 days later by inoculating sporozoites into both ceca. They found that resistance was present in the previously uninfected, ligated cecum as well as in the unligated cecum which had been exposed to the *E. tenella*. These results suggested that the immunity acquired by the ligated cecum was mediated

either by the humoral antibodies or lymphoid cells or both, although attempts to demonstrate the presence of antibodies in cecal tissue from immune chickens have failed (Horton-Smith and Long, 1963).

Challey (1962) compared the response of bursectomized (bursa of Fabricius) with nonbursectomized chickens to infection with *E. tenella*, and observed a greater mortality in the former than in the latter groups, suggesting that the host's immune response was impaired in some way. Since bursectomy resulted in a failure to develop immunity to *E. tenella*, they postulated that the protection observed in the nonbursectomized chicken could possibly be related to a higher level of gamma globulin and circulating antibodies present. In contrast, Pierce and Long (1965), using bursaless birds which were hatched from embryos inoculated *in ovo* with testosterone between six to nine days of incubation (to arrest development of the bursa of Fabricius), reported that the levels of serum globulins were greatly reduced or undetectable in spite of repeated inoculation with *E. tenella* oocysts. Nevertheless, these birds were successfully immunized, so that they resisted infection when challenged with viable oocysts of *E. tenella*. Complete surgical thymectomy was attempted within the first one and one-half hours after hatching in a second phase of the study. In spite of a significant reduction in the number of small lymphocytes in the blood, the thymectomized birds were successfully immunized against *E. tenella*. They concluded that the experiments did

not show a significant role of humoral antibodies in the mediation of resistance to E. tenella.

The measurement of antibody response to E. tenella infection in chickens remains a problem to be solved. Although numerous attempts have been made to find a satisfactory technique for the purpose, no method has been outlined which is conclusive and universally acceptable. Pierce et al. (1962) infected a number of birds with E. tenella oocysts in graded doses, and collected the sera from these birds at various intervals from seven to 63 days. When these serum samples were analyzed by electrophoresis using the Tiselius technique for quantitation of various serum fractions, no significant differences were observed in the components of the serum between the infected and the uninfected group. Schlueter (1963) used microelectrophoresis on cellulose acetate paper and standard chemical analysis methods to fractionate sera from infected birds and reported a marked reduction in the total proteins, albumin, and globulins on the fifth day after infection with a gradual rise to normal levels for albumin and an increased level for total protein and globulins by the 11th day. The sera of the infected animals showed higher levels of non-protein nitrogen during the course of infection.

Scattered reports using serological techniques to measure the antibody response are available in the literature but none of these seems to be conclusive. Pierce

et al. (1963) demonstrated precipitins in sera from part of a group of artificially infected birds, but not in others. McDermott and Stauber (1954) suggested the use of an agglutination test using merozoites as an antigen. They injected a rabbit and a rooster with a formalized suspension of merozoites and demonstrated the presence of agglutinins for at least 30 days, the maximum titers being observed between the tenth and 15th day post-infection. Herlich (1961) suggested that the presence of precipitins and neutralizing antibodies could be shown by treating the sporozoites with hyperimmune serum followed by inoculation into birds intrarectally. The sporozoites treated with hyperimmune serum gave rise to less severe infections than sporozoites treated with normal serum. The neutralizing antibodies were described to be species-specific. Long et al. (1963) reported the appearance of lysins in most of the immune sera, which could lyse sporozoites and merozoites on incubation at 37° C for one hour. The lytic activity of immune sera was destroyed by heating at 60° C for 30 minutes, but could be restored by the addition of fresh normal chicken and guinea pig serum; although guinea pig serum alone at a dilution of 1:60 showed a similar cytolytic activity. Lysins appeared eight days following an oral inoculation of E. tenella oocysts, however, the time of appearance and the intensity of lytic reaction were influenced by the size of the oocyst dose (Burns and Challey, 1965). When the sporozoites or merozoites were incubated with normal

serum under similar conditions, no lysis occurred, but the sporozoites underwent some morphological changes and exhibited a reduced infectivity.

In the present investigation, the characterization of host-response to *E. tenella* infections in chickens was accomplished by making hematological observations in conjunction with serum analysis and protection test.

MATERIALS AND METHODS

I. Experimental Design

The present investigation involved the use of 382 birds divided into two groups, the first one with 197 and the second with 185 birds.

In the first group, 56 birds were left as uninfected controls and the remaining 141 birds were orally inoculated with 500 sporulated *Eimeria tenella* oocysts. Eighty-eight of these 141 birds were superinfected with 5,000 sporulated *E. tenella* oocysts seven days after the initial inoculation. Forty-one of the 88 superinfected birds were given another superinfection with 50,000 sporulated *E. tenella* oocysts seven days post-superinfection with 5,000 oocysts. In the second group, 56 birds were left as uninfected controls, 60 inoculated with 5,000 and the remaining 69 with 50,000 sporulated *E. tenella* oocysts. Seventeen birds from each of the above-mentioned treatments in both the groups were challenged with a dose of 100,000 *E. tenella* oocysts 35 days post-treatment. Five out of the 17 birds in each treatment were used for recording per cent survival in the challenge test. Three birds from each treatment, in both the groups, were individually blood sampled for hematological observations and serum analysis. The blood sampling was done on 0, 3, 6, 9, 13, 17, 21, 28, 35, 37, 39, 42, and 45 days post-infection in all

the treatments as well as in the corresponding uninfected controls. Blood and serum samples were also collected from the challenged birds at 37, 39, 42, and 45 days, that is, two, four, seven, and ten days post-challenge. After collection of the blood and serum samples at each interval, the birds were euthanized, necropsied, and the cecal mucosa examined for gross pathology. In addition, the droppings of birds in each treatment series were examined for the presence of blood and oocysts from seven to ten days after an inoculation, before and after challenge.

II. Experimental Animals

White Leghorn cockerels (Kimber strain K-139), four weeks of age, were divided into two groups, I and II, and maintained in battery-type cages. Commercially manufactured chicken feed was given *ad libitum*, the only variation from conventional formula of ingredients being the absence of a coccidiostatic drug.

III. *Eimeria tenella* Inoculum

Oocysts were produced by a standard method (Edgar, 1961), suspended in 2 per cent potassium dichromate solution and allowed to sporulate at room temperature (25° C); based on microscopic examination, sporulation was 95 per cent complete 72 hours later. The approximate number of oocysts in the dichromate suspension was standardized by determining the total number of oocysts in a hemocytometer (Bray, 1957). Birds were inoculated by giving the de-

sired numbers of oocysts by mouth, using a 16 gauge cannula with a rubber tip, attached to a glass hypodermic syringe.

IV. Collection of Blood and Serum Samples

Blood samples were obtained by cardiac puncture, using a one and one-half inch, 20 gauge needle and a 10.0 ml disposable syringe, with ethylene diamine tetra-acetate (EDTA) as an anticoagulant. Hematologic studies were carried out within one hour of collection. For serum collection, 3.0 to 5.0 ml of blood was allowed to clot, the serum decanted and immediately frozen for future biochemical analysis.

V. Scoring of Lesions

The cecal mucus membranes were scored for the intensity of lesions as follows:

++++	Very severe; confluent hemorrhagic lesions.
+++	Severe; large, isolated ecchymotic hemorrhages.
++	Less severe; small isolated ecchymotic hemorrhages.
+	Light; small petechial hemorrhages.
-	No lesions.

VI. Total Leukocyte Count

A modification of Rees-Ecker (Rees and Ecker, 1923) method was employed. For diluting fluid, stock solution of brilliant cresyl blue (sodium citrate, 3.8 grams; brilliant cresyl blue, 0.5 grams; Ringer's solution, 100.0

ml) was prepared and stored in the refrigerator at 4° C. Immediately before use, the stock solution was filtered and diluted 1:10 with Ringer's solution. Using a standard Sahli pipette, 0.02 ml of blood was mixed with 3.0 ml of the diluted brilliant cresyl blue solution, mixed well, and allowed to stand for one hour at room temperature. One of the counting chambers of the hemocytometer was filled with the diluted suspension and counted, using the high dry objective (440 x). It was necessary to remove the top lens of the microscope substage condenser and reduce the amount of light passing through the specimen. By this method, leukocytes were readily seen to be stained dark violet as compared to the refractile light green or light blue thrombocytes with or without specific granules. By counting the four large corner squares and multiplying by a dilution factor of 378, the total number of leukocytes (WBC) per cubic millimeter was determined.

VII. Differential Leukocyte Count

Blood films were prepared by a standard method (Bray, 1957) and stained according to Mukkur and Bradley (1967), which involved fixing in methyl alcohol for five minutes and staining with diluted Giemsa's stain (1:50 with distilled water, pH 7.2) for eight hours. After completion of the staining period, the slides were washed with tap water, blotted dry and examined, using an oil immersion lens (970 x). A total of 100 leukocytes were counted, and percentage of polymorphonuclear cells (PMN cells),

monocytes, and lymphocytes were noted, using the criteria of Lucas and Jamroz (1961) for identification of different types of cells. Due to the difficulty in distinguishing between heterophils and eosinophils and the infrequency of the occurrence of basophils, these two types were counted along with the basophils as polymorphonuclear cells. Small lymphocytes were distinguished from the nucleated thrombocytes according to the method of Mukkur and Bradley (1967).

VIII. Packed Cell Volume (PCV)

A microtechnique using heparinized capillary tubes was employed. Each tube was filled with blood, sealed at one end with plastic clay and centrifuged at 11,500 rpm for five minutes in a Model MB centrifuge.[1] At the completion of the cycle, the tubes were placed in a microcapillary tube reader[2] and the packed erythrocyte column measured as PCV per cent (%) for each sample.

IX. Hemoglobin Determination

The cyanmethemoglobin method was employed. In this technique (Frankel *et al.*, 1963), 5.0 ml of cyanmethemoglobin reagent[3] was mixed with 0.02 ml of blood,

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[1]International Equipment Company, Needham Heights, Massachusetts.

[2]*Ibid.*

[3]Hycel Research Products, Hycel, Inc., Houston, Texas.

allowed to stand at room temperature for 30 minutes, and centrifuged at 3,000 rpm for 15 minutes (to pack the cell stroma). The optical density of the supernatant fluid was measured in a spectrophotometer[4] at a wavelength of 540 millimicrons and the reading converted into grams of hemoglobin per 100 ml (Hb. grams %) of blood using a standard curve (Figure 1).

X. Total Protein

The method of Weichselbaum (1946), with slight modifications, was employed. A standard curve was first determined by using a 10 per cent solution of bovine serum albumin (Figure 2). For the unknown samples, 0.1 ml of serum was mixed with 8.0 ml of stable biuret reagent,[5] incubated at 37° C for 45 minutes, and per cent transmission recorded at a wave length of 540 millimicrons using a spectrophotometer.[6] The concentration of protein was read directly from the standard curve and expressed as grams of protein per 100 ml of serum.

XI. Electrophoresis

The technique utilized a Microzone[R] electrophoresis cell (Beckman Model R-101).[7] In this method, 0.50 microliters of the serum sample was applied to a cellulose

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[4]Bausch and Lomb Spectronic 20.

[5]Hycel Research Products, Hycel, Inc., Houston, Texas.

[6]Bausch and Lomb Spectronic 20.

[7]Beckman Instruments, Inc., 2500 Harbor Boulevard, Fullerton, California.

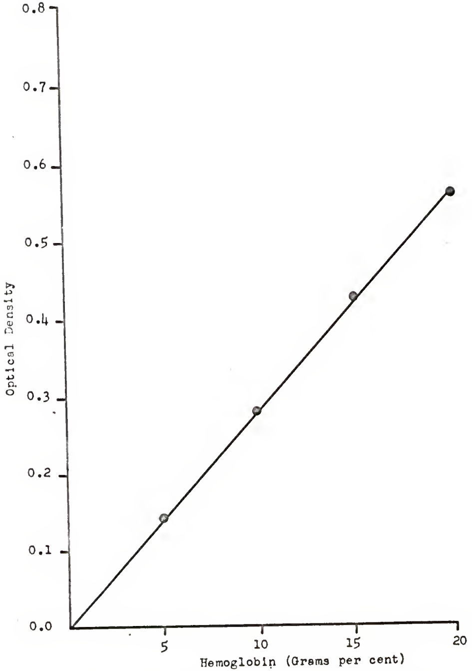

Fig. 1.--Standard curve for hemoglobin determination.

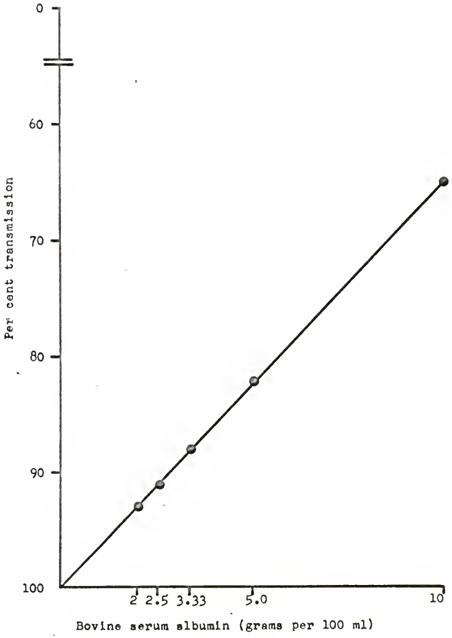

Fig. 2.--Standard curve for total protein determination.

polyacetate membrane and electrophoresis carried out using a pH 8.6 barbiturate buffer of 0.075 ionic strength at 300 volts for 35 minutes. The membrane was then stained with Ponceau S stain for ten minutes, rinsed in 5 per cent acetic acid, followed by a rinse in 90 per cent ethanol for one minute and clearing for one minute in a solution composed of 20 per cent glacial acetic acid (one part), and 90 per cent ethanol (three parts). The membrane was mounted on a glass slide and dried in a hot air oven at 100° C. The percentage of albumin and globulin fractions were obtained by scanning each membrane in a densitometer (Beckman Model R-110),[8] which gave a direct paper tracing. Relative percentages of each fraction were then calculated from the resulting graphic figure.

XII. Challenge Test

Five birds from each of the infected or immunized groups were challenged with 100,000 *E. tenella* oocysts administered orally, observed up to ten days and the survival percentage recorded. The surviving birds were euthanized and the cecal mucus membranes were scored for the intensity of lesions.

XIII. Statistical Analysis

Dunnett's multiple comparison test (1964) was carried out by the use of computer to obtain the "t" values. The variables compared included PCV per cent, hemoglobin

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[8] Ibid.

values, total WBC, polymorphonuclear cells, monocytes, and lymphocytes from hematological studies, and total protein, albumin, alpha-1, alpha-2, beta, and gamma globulin fractions obtained by electrophoresis of serum samples. The following comparisons were made: (1) uninfected controls versus treatment series, (2) challenged uninfected controls versus challenged treatment series, and (3) challenged series versus unchallenged series.

RESULTS

Hematological Observations

(A) Packed Cell Volume (PCV)

Group I: Infection of birds with 500, 500 + 5,000, or 500 + 5,000 + 50,000 *E. tenella* oocysts did not result in a significantly different PCV (P = 0.01) at any of the intervals from 0 to 35 days when compared with uninfected control birds (Tables 1-13, 27-29;[x] Figure 3). While the birds belonging to 500 and 500 + 5,000 + 50,000 treatment series exhibited a statistically significant increase (P = 0.01) 45 days post-treatment as compared to the uninfected control birds, those of 500 + 5,000 treatment series showed only an observable increase (Tables 13, 27, 29).

Challenge of the birds 35 days post-treatment resulted in a statistically significant fall in PCV (P = 0.01) in the uninfected control group (Tables 12, 31; Figure 4) on the seventh day following challenge, i.e., 42 days after the initial inoculation with *E. tenella* oocysts. The PCV values on the tenth day after challenge were lower as compared to the uninfected controls and

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[x]All references to tables, other than to the textual table 1 on page 47, are to appendix tables 1-70.

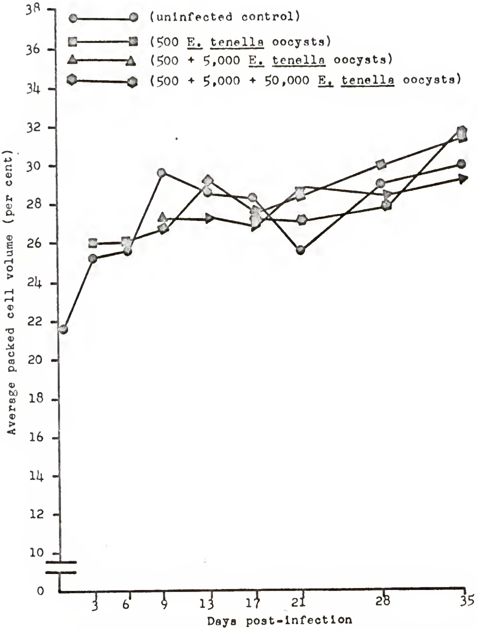

Fig. 3.--Average packed cell volume for various treatment series.

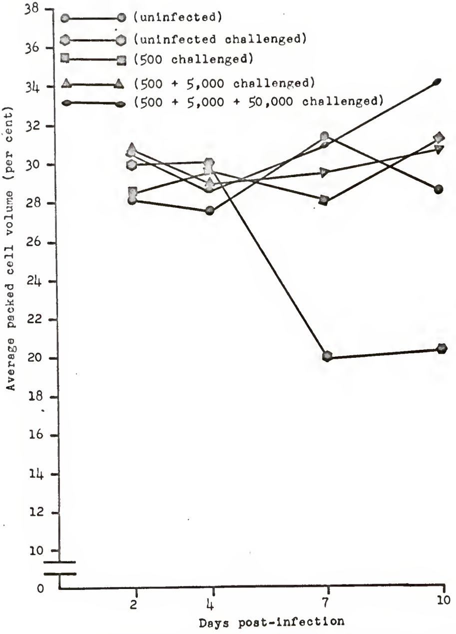

Fig. 4.--The effect of challenge infection on average packed cell volume of various treatment series.

were not statistically significant (Tables 13, 31; Figure 4). On the other hand, challenge of the birds that had been infected with 500, 500 + 5,000, or 500 + 5,000 + 50,000 E. tenella oocysts did not show significantly different values (P = 0.01) from the corresponding unchallenged birds (Tables 10-13, 31; Figure 4).

Comparison of the challenged control birds with the challenged 500, challenged 500 + 5,000, and challenged 500 + 5,000 + 50,000 revealed that the PCV drop in the challenged control birds was significantly lower (P = 0.01) as compared to the other groups (Tables 10-13, 30).

Group II: Infection of birds with 5,000 and 50,000 oocysts resulted in a statistically significant drop in PCV (P = 0.01) on the sixth day following inoculation (Tables 16, 32; Figure 5). The differences in PCV values on the ninth day after inoculation with 50,000 oocysts were statistically insignificant (Tables 17, 33; Figure 5). The PCV returned to normal values 13 days post-inoculation and stayed within this range (Tables 18-26, 32, 33; Figure 5) up to 35 days.

A statistically significant (P = 0.01) decrease in PCV was observed after challenge of uninfected control birds at seven and ten days post-challenge as compared to the unchallenged controls, but the 5,000 and 50,000 challenged treatment series showed no statistically significant differences when compared with their corresponding 5,000 and 50,000 unchallenged treatment series (Tables 23-26, 34). Similarly, a statistically significant lower

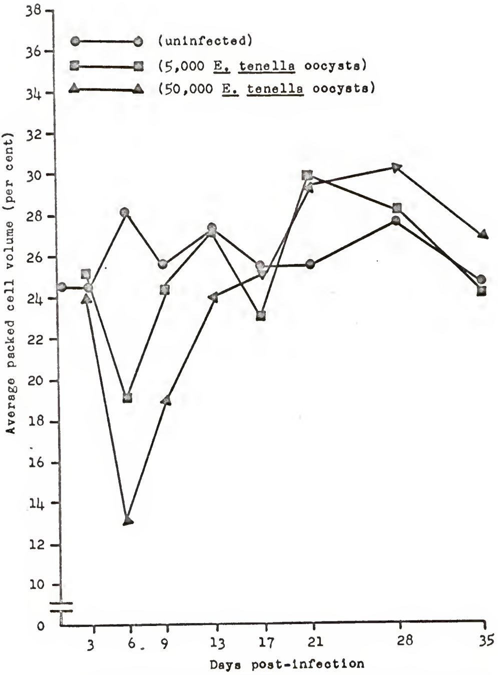

Fig. 5.--Average packed cell volume for various treatment series.

PCV was observed on the seventh and tenth day following challenge in the challenged control group when it was compared with the 5,000 challenged and 50,000 challenged groups whose values stayed within the normal range (Tables 23-26, 35; Figure 6).

(B) Hemoglobin Values

Group I: The hemoglobin values in birds infected with 500, 500 + 5,000, and 500 + 5,000 + 50,000 E. tenella oocysts did not differ significantly from the uninfected control values at any of the intervals from 0 to 35 days when tested at 0.01 level of significance (Tables 1-13, 27-29; Figure 7). When birds in these treatments were challenged on the 35th day, a statistically significant drop (P = 0.01) was observed in the challenged controls as compared to the unchallenged controls at seven and ten days post-challenge (Tables 12, 13, 31; Figure 8). No such drop was evident in 500 challenged, 500 + 5,000 challenged, and 500 + 5,000 + 50,000 challenged treatment series when compared with their corresponding unchallenged values (Tables 10-13, 31).

Group II: The hemoglobin values of birds inoculated with 5,000 E. tenella oocysts were observably lower than the uninfected controls at six days post-infection, which returned to the normal range on the ninth day (Tables 16-17; Figure 9), although these values were higher than those at three days post-infection. When 50,000 E. tenella oocysts were used as the inoculum, the hemoglobin values

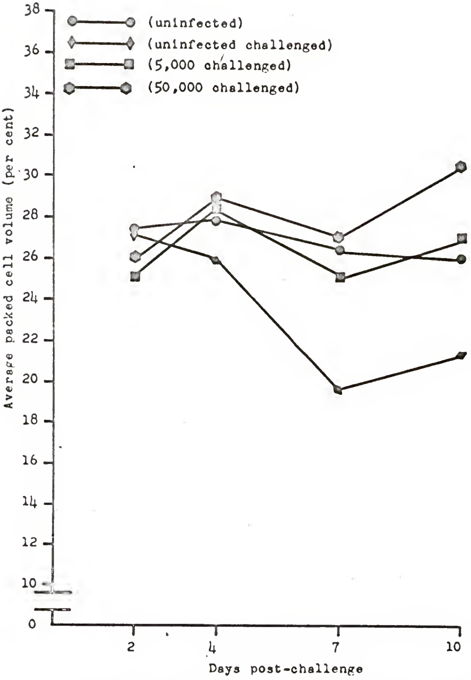

Fig. 6.--The effect of challenge infection on average packed cell volume for various treatment series.

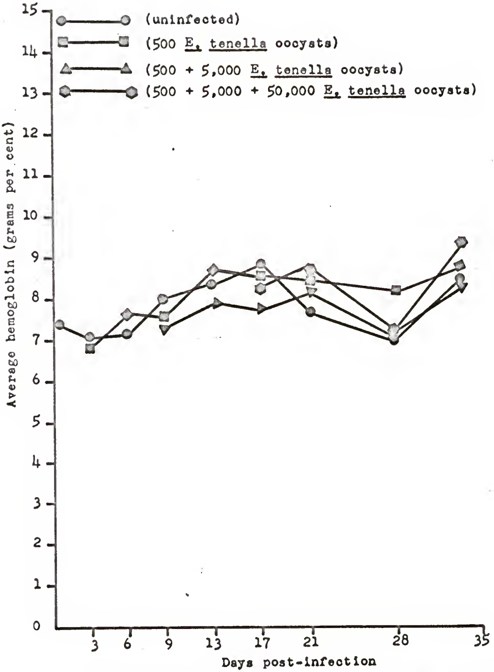

Fig. 7.--Average hemoglobin values for various treatment series.

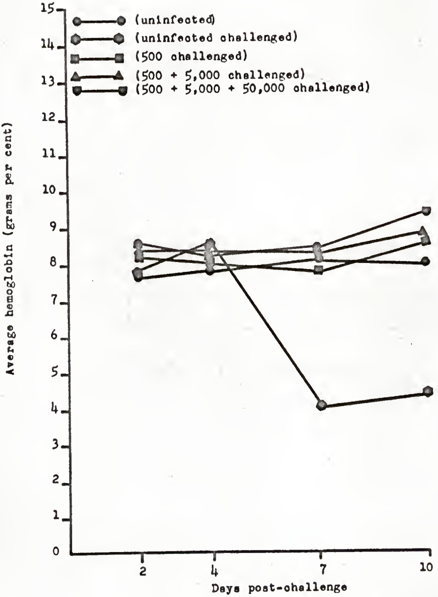

Fig.8.--The effect of challenge infection on average hemoglobin values for various treatments.

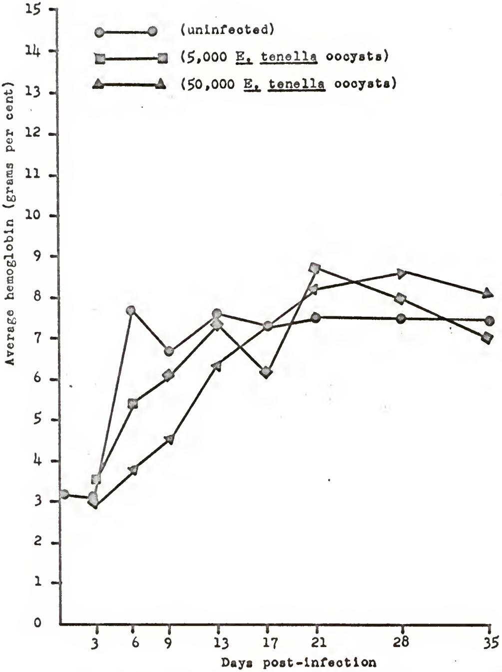

Fig. 9.--Average hemoglobin values for various treatment series.

were significantly lower (P = 0.01) on comparison with the uninfected controls six days post-infection. The hemoglobin values remained low on the ninth day as compared to the uninfected control birds, although they were not significant statistically (Tables 15-17, 33; Figure 9). These values returned to normal on the 13th day and stayed within this range up to 35 days (Tables 18-26, 33; Figure 9).

Challenge of the uninfected control birds showed a statistically significant drop in hemoglobin values at seven and ten days post-challenge. The birds that initially received 5,000 and 50,000 E. tenella oocysts did not show such a drop (Tables 23-26; Figure 10). On the other hand, in the 50,000 challenged treatment series, a significant increase in hemoglobin was observed four days post-challenge on comparison with the corresponding unchallenged treatment series (Table 24, 34). The uninfected control birds showed a statistically significant drop (P = 0.01) in the hemoglobin values on the seventh and tenth day post-challenge (P = 0.01), when compared with the 5,000 and 50,000 challenged treatment series (Table 35).

(C) White Blood Cell Response

Group I: A statistically significant increase (P = 0.01) in the number of lymphocytes was observed 13 days post-infection with 500 E. tenella oocysts (Tables 5, 27), but the total number of white blood cells, polymorphonuclear cells, and monocytes stayed within the normal

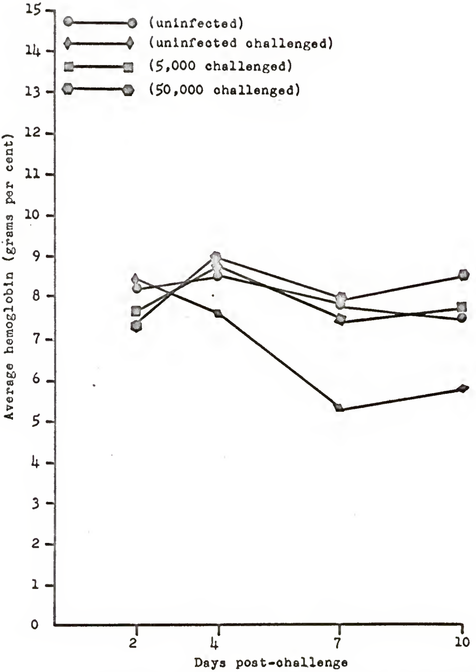

Fig. 10.--The effect of challenge on average hemoglobin values for various treatment series.

range up to 35 days (Tables 1-9, 27). Superinfection with 5,000 E. tenella oocysts resulted in an increase in total white blood cells with a concurrent increase in the number of lymphocytes six days following superinfection, i.e., on the 13th day (Tables 5, 28). However, superinfection of birds that had received 500 + 5,000 E. tenella oocysts with 50,000 oocysts did not result either in an increase or decrease in the number of total white blood cells, polymorphonuclear cells, monocytes, or lymphocytes (Tables 1-14, 29).

No changes in the above variables could be discerned on challenge of the uninfected control, and 500 + 5,000 treated birds (Tables 10, 12, 13, 30), except that 500 + 5,000 challenged birds showed a statistically significant increase (P = 0.01) in the number of polymorphonuclear cells and monocytes four days post-challenge (Tables 11, 30). Comparison of the challenged control birds and challenged 500 treatment series with their corresponding unchallenged treatments revealed an increase in the number of total white blood cells two days post-challenge (Tables 10, 31). On the other hand, an increase in the number of polymorphonuclear cells was noticed two days post-challenge (Tables 10, 31) in 500 + 5,000 + 50,000 challenged birds.

Group II: No statistically significant increase (P = 0.01) in the total number of white blood cells, polymorphonuclear cells, monocytes, and lymphocytes were observed following an infection with either 5,000 or

50,000 E. tenella oocysts (Tables 14-22, 32, 33).

Challenge of these birds in the above treatments on the 35th day post-infection did not cause any shift in the cell populations (Tables 23-26, 34, 35). Because of the observed biological variation and the resulting lack of uniformity and regularity in the patterns of cell changes, it was concluded that the cell response in birds infected or superinfected with E. tenella oocysts, if any, was not detectable by the techniques used in this investigation.

Gross Pathology of Initial Infections

Group I: Inoculation of birds with 500 sporulated E. tenella oocysts did not cause any deaths, but a few petechial hemorrhages were seen on the cecal mucosa at necropsy at six days post-infection. No blood was observed in the droppings, although a small number of oocysts was detected by fecal examination on the seventh day after inoculation. Superinfection of birds in the 500 treatment series with 5,000 E. tenella oocysts, one week later, resulted in no change in the degree of hemorrhagic lesions on the cecal mucosa and there were no deaths. Blood was not seen in the droppings but a few oocysts were detected by fecal examination on the seventh post-superinfection day. Superinfection with 50,000 E. tenella oocysts resulted neither in mortality nor was there an increase in the severity of the lesions; neither blood nor oocysts could be detected in the droppings seven days later.

Group II: Inoculation of birds with 5,000 *E. tenella* oocysts resulted in 11 per cent mortality seven to ten days later. Blood was detected in the droppings from four to seven days post-inoculation and large isolated hemorrhagic lesions were observed on the cecal mucosa at necropsy. The surviving birds recovered and appeared healthy, but *E. tenella* oocysts were detected up to ten days post-inoculation.

Inoculation of birds with 50,000 *E. tenella* oocysts resulted in 23 per cent mortality. Confluent hemorrhagic lesions were seen at necropsy on the cecal mucosa and many of the ceca were filled with clotted blood. Frequently, cecal cores composed of clotted blood and desquamated cell debris were seen inside the ceca, some of which were passed in the droppings. Blood was detected in the droppings from four to seven days post-inoculation and oocysts were detected in the droppings from seven to ten days following inoculation. Birds that recovered appeared normal.

Gross Pathology Due to Challenge Infection

Group I: The ceca of the challenged control (uninoculated) birds were filled with blood four days post-challenge which was discharged in the droppings. Hemorrhagic lesions were observed on the cecal mucosa at necropsy, seven days post-challenge and *E. tenella* oocysts were detected in the droppings from seven to ten days post-challenge.

In birds belonging to the 500 challenged treatment series, both blood and oocyst discharge were detected in the droppings on the seventh day post-challenge and the cecal mucosa of representatives from this group showed confluent hemorrhagic lesions at necropsy. Birds in the 500 + 5,000 treatment series showed small petechiae on the cecal mucosa, but no free blood; E. tenella oocysts were detected in the droppings on the seventh day after challenge. Birds in the 500 + 5,000 + 50,000 challenged treatment series had normal-appearing cecal mucosa at necropsy. Neither blood nor oocysts were detected in the droppings seven days after challenge.

Group II: The challenged control birds (uninoculated) showed a similar picture to that in group I.

Birds of the 5,000 challenged treatment series did not pass blood, but oocysts were detected in the droppings seven days after challenge.

A few petechiae were seen on the cecal mucosa but in the 50,000 treatment series, neither blood nor oocysts were seen in the feces and no cecal lesions were present.

Protection Test

Five birds from each of the treatment series in groups I and II were challenged with 100,000 sporulated E. tenella oocysts 35 days post-treatment. The number of birds surviving up to ten days post-challenge was recorded and the per cent protection calculated (Table 1).

TABLE 1

EFFECT OF CHALLENGE INFECTION ON UNINFECTED CONTROLS AND VARIOUS TREATMENT SERIES

Group	Treatment	No. of birds surviving/No. of birds used	Per Cent Survival	Cecal Lesions in Survivors[x]
I	Challenged Uninfected Controls	2/5	40	++++ (2)
	500 Challenged	4/5	80	++++ (1) +++ (3)
	500 + 5,000 Challenged	5/5	100	++ (5)
	500 + 5,000 + 50,000 Challenged	5/5	100	- (5)
II	Challenged Uninfected	2/5	40	++++ (2)
	5,000 Challenged	5/5		++ (5)
	50,000 Challenged	5/5	100	- (5)

[x]The number in parentheses denotes the number of birds.

While challenge of the uninfected controls in both groups I and II resulted in 60 per cent mortality, only 20 per cent of the birds died in the 500 treatment series. There was no mortality in birds from the 5,000, 500 + 5,000, 50,000, and 500 + 5,000 + 50,000 treatment series.

Serum Analysis

Group I: No statistically significant differences (P = 0.01) were detected in the total protein, albumin, and alpha-1, alpha-2, beta, and gamma globulin values of sera from birds inoculated with 500, 500 + 5,000, or 500 + 5,000 + 50,000 *E. tenella* oocysts when compared with similar analyses of the sera from the uninfected controls, 0 to 28 days following inoculation (Tables 36-43, 62-64). Birds in the 500 treatment series showed a significant decrease (P = 0.01) in gamma globulin at 35 days and in alpha-1 globulin levels at 42 days post-inoculation (Tables 44, 47, 62). In the 500 + 5,000 treatment series, a significant increase (P = 0.01) in gamma globulin levels at 37 days and a decrease (P = 0.01) at 39 days was observed.

In addition, a significant decrease in alpha-1 globulin levels at 42 days was observed in 500 + 5,000 treatment series (Tables 45, 47, 63). A significant drop (P = 0.01) in gamma globulin values was observed at 35 and 42 days post-treatment with 500 + 5,000 + 50,000 *E. tenella* oocysts (Tables 44, 45, 47, 64). Comparison of the challenged controls with challenged 500, 500 + 5,000, and 500 + 5,000 + 50,000 treatment series revealed no

significant differences (Tables 45-48, 65). Comparison of the challenged uninfected controls with the corresponding unchallenged controls revealed a statistically significant decrease (P = 0.01) in the levels of total protein and gamma globulins at four and seven days (Tables 46, 47, 66) and in alpha-1 globulin at seven days post-challenge (Tables 47, 66), but albumin, alpha-2, and beta globulins did not exhibit such differences (Tables 45-48, 66). On the other hand, with the challenged 500 treatment series, total protein, beta, and gamma globulin values dropped significantly (P = 0.01) at four days post-challenge when compared with the corresponding unchallenged series (Tables 46, 66). Similarly, a significant decrease (P = 0.01) in gamma globulin was observed at four days post-challenge in 500 + 5,000 treatment series on comparison with the corresponding unchallenged series (Tables 46, 66). No statistically significant differences were detected in total protein, albumin, or any of the globulins between the challenged and unchallenged 500 + 5,000 + 50,000 treatment series (Tables 45-48, 66).

<u>Group II:</u> No significant differences were revealed in any of the variables by inoculation of birds with 5,000 <u>E. tenella</u> oocysts (Tables 49-57, 67). However, inoculation with 50,000 <u>E. tenella</u> oocysts resulted in a significant drop (P = 0.01) in total protein and albumin at three days which continued at a low level until six days, but returned to within normal range on the ninth day (Tables 50, 51, 68; Figures 11, 12). A significant

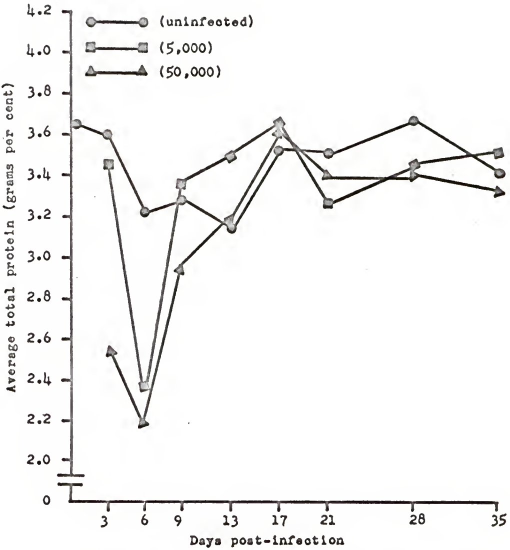

Fig. 11.--Average total protein for various treatment series.

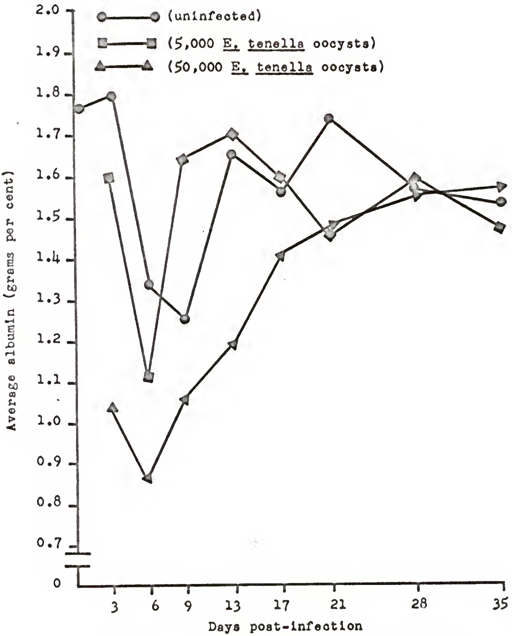

Fig. 12.--Average albumin values for various treatment series.

decrease (P = 0.01) in the levels of alpha-1 and gamma globulin levels was observed in 5,000 treatment series at 42 days and 50,000 treatment series at 39 days post-inoculations (Tables 58, 60, 67, 68). A statistically significant drop (P = 0.01) was observed in the gamma globulin levels at four days and albumin at ten days in the challenged controls when compared with 5,000 treatment series, and a similar drop in gamma globulin at four days on comparison with the 50,000 challenged treatment series (Tables 59-61, 69). In addition, there seemed to be a general trend towards lower values for total protein, alpha-1, beta, and gamma globulin levels in challenged uninfected control series in comparison to challenged 5,000 and 50,000 treatment series (Tables 59-61, 69). When the challenged uninfected control series was compared with the corresponding unchallenged series, a significant reduction (P = 0.01) in total protein at four and seven days, alpha-1 globulin at seven days, and beta and gamma globulins at four days after challenge, was detected (Tables 59, 60, 70). No such differences were revealed on comparing 5,000 and 50,000 treatment series with the corresponding unchallenged series (Tables 58-61, 70).

DISCUSSION

(A) Hematological Observations

This investigation made it evident that heavy *E. tenella* infections produced a drop in PCV which was shown to be dependent on the number of oocysts used for infection. When a single dose of 50,000 *E. tenella* oocysts was used for infection, the drop in PCV was maximum at six days and continued at low levels in comparison to the control values at nine days, returning to values within the normal range at 13 days post-infection (Figure 5), thus confirming the observation of Natt and Herrick (1955). Although the drop in PCV was also maximum at six days when 5,000 *E. tenella* oocysts were used for infection, it returned to normal values at nine days post-infection (Figure 5). No such decrease in PCV was encountered when the birds were infected with 500 *E. tenella* oocysts only (Figure 3). The drop in PCV in the infected birds was accompanied by lower values for hemoglobin during the course of infection, when compared with the uninfected controls (Figure 9). Hence, it was concluded that the drop in PCV and hemoglobin values could be used as an indicator of the severity of infection, which, in turn depended on the number of oocysts used for infection. This conclusion was further substantiated by the fact that while

infection with 50,000 _E. tenella_ oocysts produced 23 per cent mortality and (++++) order cecal lesions, 5,000 oocysts resulted in only 11 per cent mortality and (+++) order cecal lesions (Table 1). A dose of 500 _E. tenella_ oocysts did not cause any mortality and only (+) order cecal lesions (Table 1).

If the birds had been previously exposed to _E. tenella_ oocysts, they responded in a different manner to subsequent infections or superinfections. This was illustrated by the fact that while single infections of the previously unexposed birds with 5,000 and 50,000 _E. tenella_ oocysts resulted in a drop in PCV, hemoglobin values and mortality (Figures 5, 9; Table 1), superinfection of birds that had received 500 _E. tenella_ oocysts, as in group I, with 5,000 oocysts or a superinfection of birds in the 500 + 5,000 treatment series with 50,000 _E. tenella_, produced neither a drop in PCV and hemoglobin values nor any mortality (Figure 3; Table 1).

It was also evident from the data that the immunity status of the birds, as judged by resistance to a challenge infection with 100,000 _E. tenella_ oocysts, was dependent on the amount of antigen (number of _E. tenella_ oocysts), that the bird had previously received, and could be related to changes in PCV, hemoglobin values, and per cent mortality. For instance, while challenge of the uninfected control birds, in both groups I and II, resulted in lower values for PCV and hemoglobin values at seven and ten days post-challenge (Figures 4, 6) with 60 per

cent mortality (Table 1), challenge of those in the 500 treatment series showed only a slight drop in PCV at seven days post-challenge (Figure 4) accompanied by 20 per cent mortality (Table 1). On the other hand, challenge of birds that had initially been exposed to or infected with at least 5,000 E. tenella oocysts resulted in no drop in PCV and hemoglobin values (Figures 4, 6) and no mortality, such as in 5,000, 500 + 5,000, 50,000, and 500 + 5,000 + 50,000 treatment series (Table 1). It was thus concluded that PCV and hemoglobin values could be used as indirect indicators of the immunity status of the birds, but only after a challenge infection. The increase in PCV in 500, 500 + 5,000, and 500 + 5,000 + 50,000 treatment series 45 days post-treatment was attributed to normal variation, since this was seemingly not related to the course of E. tenella infection.

From our data on the total white blood cell and differential counts in the infected or superinfected birds, no shift in the populations of different types of cells was detectable. This finding was in contrast to the report of Natt (1959).

(B) Serum Analysis

It was concluded from studies on serum analysis that the damage inflicted to the host due to E. tenella infection, as measured by differences in the total serum protein or various fractions, was proportional to the severity of infection which was, in turn, determined

by the number of E. tenella oocysts used for single infection. While only an observable reduction in total protein was noticed at three days post-infection with 5,000 E. tenella oocysts, this reduction in total protein was accompanied by a similar reduction in albumin at three days continuing at low levels at six days post-infection with 50,000 E. tenella oocysts (Figures 11, 12). Challenge infection of control birds in both groups I and II with 100,000 E. tenella oocysts resulted in a reduction in total protein at four and seven days accompanied by a similar decrease in alpha-1 globulin at seven days and beta and gamma globulins at four days post-infection or post-challenge (Tables 46-47, 59-61, 66, 70), but in groupII, there was also a reduction in albumin levels at seven and ten days post-challenge (Tables 59-61, 70). From the above observations, it was evident that with the increase in the number of oocysts used for infection, the number of variables affected was increased. These findings were in contrast to those of Schlueter (1963) and Pierce et al. (1962).

Also, it was evident from the data that the amount of exposure, that is, the number of oocysts used for initial infection, appeared to be an important factor in how well the bird resisted these physiological changes. While infection of unexposed birds with 5,000 oocysts caused a reduction in total protein at three days post-infection (Figure 11), the same dose did not produce such a change if the birds had initially received 500 E. tenella

oocysts. Similarly, superinfection of 500 + 5,000 treatment series with 50,000 E. tenella oocysts produced no significant changes in the serum, whereas this dose when used for infecting a previously unexposed bird by itself produced reduction in total protein and albumin at three and six days post-infection (Figures 11, 12). Results of the challenge infection pointed out that the greater the amount of exposure that the bird experienced prior to challenge infection, the more resistant it was to serum changes after the challenge infection. For example, challenge of birds in the 500 treatment series resulted in a drop in total protein, alpha-1, and gamma globulin levels at four days post-challenge (Tables 46, 66); no such changes were discernible in birds of the 5,000 treatment series (Tables 59, 70). On the other hand, only a drop in gamma globulin levels was detected in the 500 + 5,000 treatment series four days post-challenge (Tables 46, 66). But neither reduction in total serum protein nor any of its fractions was evident when birds of the 50,000 or 500 + 5,000 + 50,000 treatment series were challenged with 100,000 E. tenella oocysts (Tables 58-61, 45-48, 66, 70). Thus it was concluded that an initial exposure of birds to at least 50,000 E. tenella oocysts was essential for them to tolerate the effects of a challenge infection of 100,000 E. tenella oocysts, as measured by changes in the serum proteins or its various fractions.

No such differences either in total serum protein or its various globulin components were found between

the control or susceptible and resistant birds (as judged by the results of challenge infection) (Table 1), that were considered to be a manifestation of immune response.

However, a problem presumably of natural variations was encountered which might have affected some of our results; for instance, a significant reduction in gamma globulin levels at 35 days post-treatment in 500 and 500 + 5,000 + 50,000 treatment series (Tables 45, 63, 65). Other examples of this nature were encountered in our data.

SUMMARY AND CONCLUSIONS

(A) Hematological Observations

Single infection of birds with 5,000 or more *E. tenella* oocysts resulted in an observable or a significant drop in PCV and hemoglobin values which was maximum at six to seven days post-infection.

The drop in PCV and hemoglobin was proportional to the intensity of infection which, in turn, depended on the number of oocysts used for infection. The PCV and hemoglobin values returned within the normal range at nine and 13 days after infection with 5,000 and 50,000 *E. tenella* oocysts, respectively. This suggested that PCV and hemoglobin values could be used as an index of the severity of infection.

The decrease in PCV was accompanied by a decrease in the hemoglobin values, confirming a correlation between these two variables.

An exposure of the birds with a minimum of 5,000 *E. tenella* oocysts was essential to obtain 100 per cent survival against a challenge infection with 100,000 *E. tenella* oocysts. Since the birds that were resistant (as judged by the lack of mortality after challenge infection) also did not show either an observable or significant drop in PCV and hemoglobin values on challenge,

it was suggested that PCV and hemoglobin values could be used as indirect indicators of the immunity status of the birds, but only after challenge infection.

No significant white blood cell response was detected in infected birds before or after challenge infection that could be said to be typical of *E. tenella* infection in chickens.

(B) Serum Analysis

Single infection of birds with *E. tenella* oocysts, depending on the number used, resulted in an observable or significant reduction in the levels of total protein, alpha-1, beta, and gamma globulins within three to ten days after infection. It might or might not be accompanied by drop in albumin. Alpha-2 globulin levels remained unaffected.

Prior exposure of birds to a minimum of 50,000 *E. tenella* oocysts was considered necessary to make the bird resistant to changes due to the challenge infection in the above mentioned physiological variables.

APPENDIX

TABLE 1

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED BIRDS AT 0 DAY

Treatment Series	Uninfected			
Bird Number	1	2	3	Mean
PCV (%)	24.00	19.00	22.00	21.66
Hb. (gms. %)	8.60	6.40	7.20	7.40
Total WBC (count x 378)	30.00	22.00	4.00	18.66
PMN cells[x]	8.70	6.60	0.76	5.35
Monocytes[x]	1.20	1.98	0.20	1.13
Lymphocytes[x]	20.10	13.42	3.04	12.18

[x]Represents absolute values calculated from the total WBC.

TABLE 2

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL AND 500 TREATMENT SERIES AT 3 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
PCV (%)	26.00	25.00	25.00	25.33	23.00	24.00	31.00	26.00
Hb. (gms. %)	7.20	6.80	7.20	7.06	7.20	7.20	6.40	6.93
Total WBC (count x 378)	30.00	14.00	11.00	18.33	19.00	22.00	25.00	22.00
PMN cells^x	6.00	3.50	2.86	4.12	4.94	3.74	8.75	5.81
Monocytes^x	2.40	0.70	1.10	1.40	2.28	3.96	2.75	2.99
Lymphocytes^x	21.60	9.80	7.04	12.81	11.78	14.30	13.50	13.20

^{x}Represents absolute values calculated from the total WBC.

TABLE 3

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL AND 500 TREATMENT SERIES AT 6 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
PCV (%)	26.00	26.00	25.00	25.66	26.00	27.00	25.00	26.00
Hb. (gms. %)	7.60	7.20	6.80	7.20	7.60	8.20	7.20	7.66
Total WBC (count x 378)	19.00	14.00	30.00	21.00	14.00	16.00	16.00	15.33
PMN cells[x]	2.47	2.10	3.90	2.82	4.20	2.88	4.64	3.91
Monocytes[x]	1.14	1.40	2.70	1.75	1.54	2.88	2.08	2.16
Lymphocytes[x]	15.39	10.50	23.40	16.43	8.26	10.24	9.28	9.21

[x]Represents absolute values calculated from the total WBC.

TABLE 4

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 500, AND 500 + 5,000 TREATMENT SERIES AT 9 DAYS

Treatment Series	Uninfected				500				500 + 5,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	32.00	28.00	29.00	29.66	25.00	29.00	26.50	26.83	27.00	27.00	28.00	27.33
HB. (gms. %)	8.60	7.80	7.60	8.00	7.60	7.60	7.60	7.60	7.60	7.20	7.20	7.33
Total WBC (count x 378)	30.00	40.00	38.00	36.00	50.00	50.00	44.00	48.00	40.00	38.00	50.00	42.66
PMN cells[x]	10.20	2.40	7.98	6.86	2.50	6.50	5.72	4.91	4.00	4.94	11.00	6.65
Monocytes[x]	4.50	3.60	4.18	4.09	5.00	7.50	3.08	5.19	7.20	4.94	8.00	6.71
Lymphocytes[x]	15.30	34.00	25.84	25.05	42.50	36.00	35.20	37.90	28.80	28.12	31.00	29.30

[x]Represents absolute values calculated from the total WBC.

TABLE 5

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 500, AND 500 + 5,000 TREATMENT SERIES AT 13 DAYS

Treatment Series	Uninfected				500				500 + 5,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	26.00	30.00	30.00	28.66	28.00	32.00	28.00	29.33	26.00	26.00	30.00	27.33
Hb. (gms. %)	7.20	9.00	9.00	8.40	8.20	9.40	8.60	8.73	7.20	7.60	9.00	7.93
Total WBC (count x 378)	22.00	25.00	26.00	24.33	50.00	54.00	53.00	52.33	50.00	48.00	51.00	49.66
PMN cells[x]	6.38	5.25	7.28	6.30	11.00	9.18	4.24	8.14	9.50	15.36	6.12	10.32
Monocytes[x]	3.74	0.75	2.08	2.19	2.00	8.10	7.42	5.84	1.50	5.28	2.55	3.11
Lymphocytes[x]	11.88	19.00	16.64	15.84	37.00	36.72	41.34	38.35	39.00	27.36	42.33	36.23

[x]Represents absolute values calculated from the total WBC.

TABLE 6

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 500, 500 + 5,000, AND 500 + 5,000 + 50,000 TREATMENT SERIES AT 17 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
PCV (%)	27.00	28.00	30.00	28.33	26.00	29.00	28.00	27.66
Hb. (gms. %)	8.60	9.00	9.00	8.86	8.20	9.00	8.60	8.60
Total WBC (count x 378)	30.00	40.00	22.00	30.66	50.00	38.00	19.00	35.66
PMN cells^x	9.90	10.00	5.06	8.32	8.50	3.42	4.56	5.49
Monocytes^x	2.70	2.80	3.52	3.00	2.00	4.56	1.52	2.69
Lymphocytes	17.40	27.20	13.42	19.34	39.50	30.02	12.92	27.48

TABLE 6 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
PCV (%)	26.00	26.00	29.00	27.00	26.00	28.00	29.00	27.66
Hb. (gms. %)	7.60	7.60	8.20	7.80	7.80	9.00	8.60	8.46
Total WBC (count x 378)	38.00	19.00	30.00	29.00	31.00	30.00	40.00	33.66
PMN cells[x]	5.32	1.52	7.20	4.68	9.61	9.60	17.20	12.14
Monocytes[x]	3.04	1.52	3.60	2.72	4.03	3.00	4.00	3.67
Lymphocytes[x]	29.64	15.96	19.20	21.60	17.63	17.40	18.80	17.85

[x]Represents absolute values calculated from the total WBC.

TABLE 7

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 500, 500 + 5,000, AND 500 + 5,000 + 50,000 TREATMENT SERIES AT 21 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
PCV (%)	25.00	23.00	29.00	25.66	28.50	30.00	27.00	28.50
Hb. (gms. %)	8.20	6.40	8.60	7.73	9.00	9.40	7.20	8.53
Total WBC (count x 378)	50.00	50.00	54.00	51.33	54.00	50.00	54.00	52.66
PMN cells^x	20.50	16.00	9.72	15.41	22.68	13.00	16.20	17.29
Monocytes^x	8.50	9.00	9.18	8.89	5.94	10.00	9.72	8.55
Lymphocytes^x	21.00	25.00	35.10	27.03	25.38	27.00	28.08	26.82

TABLE 7 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
PCV (%)	28.00	30.00	28.50	28.83	29.00	25.00	28.00	27.33
Hb. (gms. %)	8.20	8.20	8.20	8.20	9.00	8.20	9.00	8.73
Total WBC (count x 378)	50.00	54.00	55.00	53.00	60.00	55.00	55.00	56.66
PMN cells[x]	19.00	17.82	14.85	17.22	16.80	16.50	8.80	14.03
Monocytes[x]	12.00	10.80	9.35	10.72	12.00	9.35	14.30	11.88
Lymphocytes[x]	19.00	25.38	30.80	25.06	31.20	29.50	31.90	30.75

[x]Represents absolute values calculated from the total WBC.

TABLE 8

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 500, 500 + 5,000, AND 500 + 5,000 + 50,000 TREATMENT SERIES AT 28 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
PCV (%)	29.00	29.00	29.00	29.00	30.00	32.00	30.00	30.06
Hb. (gms. %)	7.60	6.40	7.20	7.06	8.60	7.80	7.20	8.20
Total WBC (count x 378)	70.00	72.00	73.00	71.66	68.00	60.00	69.00	65.66
PMN cells[x]	16.80	22.32	13.87	17.66	20.40	21.60	20.70	20.90
Monocytes[x]	4.90	7.20	5.84	5.98	8.16	10.80	4.83	7.93
Lymphocytes[x]	48.30	42.48	53.29	48.02	39.44	27.60	43.47	36.83

TABLE 8 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
PCV (%)	28.50	29.50	27.50	28.50	28.00	28.00	28.00	28.00
Hb. (gms. %)	6.80	7.80	6.40	7.00	6.80	6.80	7.60	7.06
Total WBC (count x 378)	71.00	70.00	75.00	72.00	80.00	78.00	71.00	76.33
PMN cells[x]	4.26	27.30	3.00	11.52	8.00	2.34	19.17	9.84
Monocytes[x]	11.36	10.50	6.75	9.67	11.20	4.68	7.10	7.66
Lymphocytes[x]	55.38	32.20	65.25	50.94	60.80	70.98	44.73	58.83

[x]Represents absolute values calculated from the total WBC.

TABLE 9

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 500, 500 + 5,000, AND 500 + 5,000 + 50,000 TREATMENT SERIES AT 35 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
PCV (%)	31.00	29.50	29.50	30.00	30.00	31.00	33.50	31.50
Hb. (gms. %)	8.60	8.60	8.20	8.46	8.20	8.60	9.40	8.73
Total WBC (count x 378)	51.00	53.00	70.00	58.00	60.00	50.00	61.00	57.00
PMN cells^{x}	5.61	15.90	1.40	7.64	30.00	9.00	18.30	19.10
Monocytes^{x}	0.51	4.24	18.90	7.88	2.40	5.50	13.42	7.11
Lymphocytes^{x}	44.88	32.86	49.70	42.48	27.60	35.50	29.28	30.79

TABLE 9 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
PCV (%)	26.00	31.50	30.50	29.33	32.00	36.50	35.00	31.66
Hb. (gms. %)	8.20	7.80	9.00	8.33	9.00	9.60	9.60	9.40
Total WBC (count x 378)	55.00	54.00	60.00	56.33	70.00	63.00	50.00	61.00
PMN cells[x]	9.35	17.28	21.00	15.88	24.50	13.23	18.50	18.74
Monocytes[x]	4.95	5.94	10.80	7.23	9.10	8.82	6.50	8.14
Lymphocytes[x]	40.70	30.78	28.20	33.22	36.40	40.95	25.00	34.12

[x]Represents absolute values calculated from the total WBC.

TABLE 10

HEMATOLOGICAL OBSERVATIONS OF VARIOUS UNCHALLENGED AND CHALLENGED SERIES AT 37 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
PCV (%)	29.00	25.00	31.00	28.33	28.00	23.00	32.00	27.66
Hb. (gms.%)	7.20	7.20	8.60	7.66	7.60	6.80	9.00	7.80
Total WBC (count x 378)	60.00	46.00	50.00	52.00	62.00	61.00	60.00	61.00
PMN cells^x	10.20	7.82	16.00	11.34	13.64	6.10	16.20	11.98
Monocytes^x	7.80	3.22	16.00	9.01	3.72	6.10	5.40	5.07
Lymphocytes^x	42.00	34.96	18.00	31.65	44.64	48.80	38.40	43.95

TABLE 10 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
PCV (%)	26.50	29.50	30.00	28.66	27.00	27.00	27.00	27.00
Hb. (gms. %)	7.20	8.20	8.20	7.86	7.20	7.20	8.20	7.53
Total WBC (count x 378)	64.00	59.00	57.00	60.00	60.00	57.00	63.00	60.00
PMN cells[x]	7.04	18.88	26.22	17.38	3.00	4.56	5.04	4.20
Monocytes[x]	8.32	7.08	10.83	8.74	3.60	7.41	21.42	10.81
Lymphocytes[x]	48.64	33.04	19.95	33.88	53.40	45.03	36.54	44.99

TABLE 10 (extended)

Treatment Series	Uninfected Challenged				500 Challenged			
Bird Number	13	14	15	Mean	16	17	18	Mean
PCV (%)	29.00	30.00	31.00	30.00	26.00	32.50	27.00	28.50
Hb. (gms. %)	7.80	7.80	8.20	7.93	6.80	9.00	7.60	7.80
Total WBC (count x 378)	72.00	80.00	76.00	76.00	92.00	78.00	81.00	83.66
PMN cells^x	33.12	19.20	24.32	25.55	21.16	28.86	11.34	20.45
Monocytes^x	20.88	16.80	13.68	17.12	16.45	10.14	13.77	13.49
Lymphocytes^x	18.00	44.00	38.00	33.33	54.28	39.00	55.89	49.72

TABLE 10 (extended)

Treatment Series	500 + 5,000 Challenged				500 + 5,000 + 50,000 Challenged			
Bird Number	19	20	21	Mean	22	23	24	Mean
PCV (%)	31.00	31.00	30.00	30.66	31.50	28.00	32.00	30.50
Hb. (gms. %)	8.60	8.20	8.60	8.46	9.00	7.80	9.00	8.60
Total WBC (count x 378)	74.00	71.00	72.00	72.33	71.00	70.00	72.00	71.00
PMN cells[x]	21.46	18.46	15.12	18.35	17.75	19.60	41.76	26.37
Monocytes[x]	19.24	9.94	10.08	13.08	10.65	12.60	5.76	9.67
Lymphocytes[x]	33.30	42.60	46.80	40.90	42.60	37.80	24.48	34.96

[x]Represents absolute values calculated from the total WBC.

TABLE 11

HEMATOLOGICAL OBSERVATIONS OF VARIOUS UNCHALLENGED AND CHALLENGED SERIES AT 39 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
PCV (%)	24.00	29.50	29.50	27.66	31.00	29.00	28.00	29.33
Hb. (gms. %)	6.40	8.60	8.60	7.86	9.00	8.20	9.00	8.73
Total WBC (count x 378)	38.00	52.00	68.00	52.66	42.00	53.00	50.00	48.33
PMN cells^x	1.52	22.88	34.00	19.47	14.28	5.83	10.50	10.20
Monocytes^x	16.34	3.12	4.08	7.84	6.30	5.30	4.00	5.20
Lymphocytes^x	20.14	26.00	29.92	25.35	21.42	41.87	35.50	32.93

TABLE 11 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
PCV (%)	31.50	32.00	32.00	31.83	26.50	28.50	30.00	28.33
Hb. (gms. %)	9.00	9.40	9.40	9.26	6.80	8.20	8.60	7.86
Total WBC (count x 378)	30.00	56.00	60.00	48.66	38.00	54.00	41.00	44.33
PMN cells[x]	12.90	11.76	32.40	19.02	21.66	19.98	14.76	18.80
Monocytes[x]	2.40	6.16	4.20	4.25	3.80	7.56	6.15	5.84
Lymphocytes[x]	14.70	38.08	23.40	25.39	12.54	26.46	20.09	19.69

TABLE 11 (extended)

Treatment Series	Uninfected Challenged				500 Challenged			
Bird Number	13	14	15	Mean	16	17	18	Mean
PCV (%)	27.00	33.50	29.00	29.83	27.00	31.50	31.00	29.83
Hb. (gms. %)	6.80	9.60	8.60	8.33	6.80	9.00	8.60	8.13
Total WBC (count x 378)	42.00	66.00	78.00	62.00	64.00	57.00	58.00	59.66
PMN cells[x]	13.44	17.66	23.40	18.00	12.16	24.51	9.86	15.51
Monocytes[x]	0.84	11.22	5.46	5.84	3.84	8.55	4.06	5.48
Lymphocytes[x]	27.72	37.62	49.14	38.16	48.00	23.94	44.08	38.67

TABLE 11 (extended)

Treatment Series	500 + 5,000 Challenged				500 + 5,000 + 50,000 Challenged			
Bird Number	19	20	21	Mean	22	23	24	Mean
PCV (%)	28.00	30.50	28.50	29.00	28.00	25.00	33.00	28.66
Hb. (gms. %)	8.20	9.00	8.20	8.46	7.80	7.60	9.60	8.33
Total WBC (count x 378)	56.00	80.00	80.00	72.00	41.00	66.00	48.00	51.66
PMN cells[x]	27.44	48.00	29.60	35.01	6.15	10.56	16.80	11.70
Monocytes[x]	8.40	9.60	37.60	18.53	2.46	5.28	3.84	3.86
Lymphocytes[x]	20.16	22.40	12.80	18.45	32.39	50.16	27.36	36.63

[x]Represents absolute values calculated from the total WBC.

TABLE 12

HEMATOLOGICAL OBSERVATIONS OF VARIOUS UNCHALLENGED AND CHALLENGED SERIES AT 42 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
PCV (%)	32.00	32.50	29.50	31.33	28.00	33.50	35.50	32.33
Hb. (gms. %)	8.20	8.60	7.60	8.13	7.80	9.00	9.60	8.80
Total WBC (count x 378)	42.00	41.00	38.00	40.33	48.00	47.00	40.00	45.00
PMN cells[x]	10.50	18.86	9.50	12.95	11.52	14.10	13.60	13.07
Monocytes[x]	3.78	4.10	2.28	3.39	2.88	2.35	4.00	3.08
Lymphocytes[x]	27.72	18.04	26.22	23.99	33.60	30.55	22.40	28.85

TABLE 12 (extended)

Treatment Series	500 + 5,000				500 + 5,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
PCV (%)	28.00	28.50	34.00	30.16	31.00	31.00	26.00	29.33
Hb. (gms. %)	7.60	7.80	9.40	8.26	7.80	7.60	6.00	7.13
Total WBC (count x 378	38.00	42.00	34.00	38.00	41.00	50.00	37.00	42.66
PMN cells^{x}	2.66	2.52	8.16	4.45	14.35	13.50	9.62	12.49
Monocytes^{x}	2.66	0.84	3.06	2.19	5.33	4.00	4.81	4.71
Lymphocytes^{x}	32.68	38.64	22.78	31.36	21.32	32.50	22.57	25.46

TABLE 12 (extended)

Treatment Series	Uninfected Challenged				500 Challenged			
Bird Number	13	14	15	Mean	16	17	18	Mean
PCV (%)	24.00	18.00	18.50	20.16	30.00	27.00	27.00	28.00
Hb. (gms. %)	5.00	3.60	3.80	4.13	8.20	7.60	7.60	7.80
Total WBC (count x 378)	38.00	40.00	43.00	40.33	51.00	46.00	38.00	45.00
PMN cells[x]	14.44	20.00	3.01	12.48	9.69	17.48	19.38	15.52
Monocytes[x]	1.90	10.00	6.02	5.97	8.16	6.90	3.80	6.29
Lymphocytes[x]	21.66	10.00	33.97	21.88	33.15	21.62	14.82	23.19

TABLE 12 (extended)

Treatment Series	500 + 5,000 Challenged				500 + 5,000 + 50,000 Challenged			
Bird Number	19	20	21	Mean	22	23	24	Mean
PCV (%)	27.00	32.00	29.50	29.50	30.00	29.00	34.00	31.00
Hb. (gms. %)	7.60	9.00	7.80	8.13	8.20	7.80	9.40	8.46
Total WBC (count x 378)	44.00	46.00	34.00	41.33	40.00	50.00	35.00	41.66
PMN cells[x]	2.20	8.74	7.48	6.14	6.80	8.00	17.85	10.88
Monocytes[x]	1.32	4.14	2.38	2.61	2.80	4.50	5.95	4.42
Lymphocytes[x]	40.48	33.12	24.14	32.58	30.40	37.50	11.20	26.36

[x]Represents absolute values calculated from the total WBC.

TABLE 13

HEMATOLOGICAL OBSERVATIONS OF VARIOUS UNCHALLENGED AND CHALLENGED SERIES AT 45 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
PCV (%)	26.00	34.00	25.50	28.50	33.00	33.00	38.00	34.66
Hb. (gms. %)	7.60	9.40	7.20	8.06	9.00	9.40	9.60	9.33
Total WBC (count x 378)	38.00	40.00	42.00	40.00	46.00	42.00	50.00	46.00
PMN cells[x]	11.02	12.40	10.50	11.31	7.36	21.84	12.50	13.90
Monocytes[x]	2.28	5.20	7.98	5.15	2.76	3.36	11.00	5.71
Lymphocytes[x]	24.70	22.40	23.52	23.54	35.88	16.80	26.50	26.39

TABLE 13 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
PCV (%)	32.00	34.00	32.00	32.66	34.50	35.00	34.50	34.66
Hb. (gms. %)	9.00	9.40	9.00	9.13	9.40	9.60	9.40	9.46
Total WBC (count x 378)	40.00	34.00	41.00	38.33	42.00	34.00	51.00	42.33
PMN cells[x]	6.40	13.60	13.12	11.04	12.60	12.92	11.22	12.25
Monocytes[x]	6.00	1.02	4.92	3.98	1.68	5.78	5.10	4.18
Lymphocytes[x]	27.60	19.38	22.96	23.31	27.72	15.30	34.68	25.90

TABLE 13 (extended)

Treatment Series	Uninfected Challenged				500 Challenged			
Bird Number	13	14	15	Mean	16	17	18	Mean
PCV (%)	25.00	19.00	26.00	23.33	32.00	31.00	31.00	31.33
Hb. (gms. %)	4.60	3.60	5.00	4.40	9.00	8.60	8.60	8.73
Total WBC (count x 378)	43.00	46.00	34.00	41.00	50.00	42.00	38.00	43.33
PMN cells^{x}	14.62	3.22	10.20	9.35	10.00	16.80	19.76	15.51
Monocytes^{x}	2.58	11.04	6.80	6.80	8.00	5.04	6.84	6.63
Lymphocytes^{x}	25.80	31.74	17.00	24.85	32.00	20.16	11.40	21.19

TABLE 13 (extended)

Treatment Series	500 + 5,000 Challenged				500 + 5,000 + 50,000 Challenged			
Bird Number	19	20	21	Mean	22	23	24	Mean
PCV (%)	33.50	28.00	31.00	30.83	33.50	33.50	35.00	34.00
Hb. (gms. %)	9.40	7.80	9.00	8.73	9.40	9.40	9.60	9.46
Total WBC (count x 378)	51.00	46.00	40.00	45.66	38.00	40.00	46.00	41.33
PMN cells[x]	13.26	11.04	13.60	12.63	19.76	16.00	10.12	15.29
Monocytes[x]	5.10	2.30	3.20	3.53	1.90	3.20	9.20	4.77
Lymphocytes[x]	32.64	32.66	23.20	29.50	16.34	20.80	26.68	21.27

[x]Represents absolute values calculated from the total WBC.

TABLE 14

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED BIRDS AT 0 DAY

Treatment Series	Uninfected			
Bird Number	1	2	3	Mean
PCV (%)	28.00	20.50	25.00	24.50
Hb. (gms. %)	3.80	2.20	3.60	3.20
Total WBC (count x 378)	8.00	14.00	13.00	11.66
PMN cells[x]	1.36	2.80	1.95	2.03
Monocytes[x]	0.48	1.54	0.52	0.85
Lymphocytes[x]	6.16	9.66	10.53	8.78

[x]Represents absolute values calculated from total WBC.

TABLE 15

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 3 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	29.50	19.00	25.00	24.50	25.00	24.50	25.00	24.83	23.00	21.00	29.00	24.33
Hb. (gms. %)	4.00	1.40	3.80	3.06	3.20	3.00	3.60	3.26	2.40	2.20	4.60	3.06
Total WBC (count x 378)	9.00	9.00	13.00	10.33	14.00	16.00	22.00	17.33	24.00	11.00	16.00	17.00
PMN cells[x]	2.07	0.63	3.38	2.03	4.62	2.72	8.80	5.38	4.08	0.77	4.32	3.06
Monocytes[x]	1.80	0.63	1.69	1.37	0.98	3.20	1.32	1.83	1.68	1.21	1.92	1.60
Lymphocytes[x]	5.13	7.74	7.93	6.93	8.40	10.80	11.88	10.12	18.24	9.02	9.76	12.34

[x]Represents absolute values calculated from the total WBC.

TABLE 16

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 6 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	28.50	27.00	29.00	28.16	24.50	9.00	23.50	19.00	11.00	9.50	19.00	13.16
Hb. (gms. %)	7.80	7.60	7.80	7.73	7.20	2.40	6.80	5.46	3.20	2.80	5.40	3.80
Total WBC (count x 378)	14.00	9.00	14.00	12.33	19.00	10.00	12.00	13.66	16.00	25.00	38.00	26.33
PMN cells[x]	2.66	3.15	4.90	3.57	7.79	2.60	4.20	4.86	8.48	4.50	10.26	7.74
Monocytes[x]	1.82	0.72	3.78	2.11	3.61	1.40	3.00	2.67	3.20	1.00	7.22	3.81
Lymphocytes	9.52	5.13	5.32	6.66	7.60	6.00	4.80	6.13	4.32	19.50	20.52	14.78

[x]Represents absolute values calculated from the total WBC.

TABLE 17

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 9 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	29.00	24.50	24.00	25.83	23.00	28.00	22.00	24.33	22.50	20.00	14.50	19.00
Hb. (gms. %)	8.20	6.00	6.00	6.73	5.00	7.60	5.80	6.13	5.80	5.00	3.00	4.60
Total WBC (count x 378)	22.00	13.00	11.00	15.33	15.00	30.00	11.00	18.66	22.00	13.00	6.00	13.66
PMN cells[x]	7.70	3.64	0.22	3.85	1.65	8.10	2.64	4.13	10.56	3.38	1.92	5.28
Monocytes[x]	2.86	0.91	6.60	3.46	1.80	4.50	2.64	2.98	2.86	0.65	0.24	1.25
Lymphocytes[x]	11.44	8.45	4.18	8.02	11.55	17.40	5.72	11.55	8.58	8.97	3.84	7.13

[x]Represents absolute values calculated from the total WBC.

TABLE 18

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 13 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	28.00	30.00	24.00	27.33	28.00	26.00	28.00	27.33	19.00	27.00	26.00	24.00
Hb. (gms. %)	7.80	8.20	6.80	7.60	7.80	6.80	7.80	7.46	5.00	7.20	7.20	6.42
Total WBC (count x 378)	18.00	20.00	8.00	15.33	30.00	12.00	18.00	20.00	15.00	15.00	37.00	22.33
PMN cells[x]	8.64	8.00	2.56	6.40	15.00	5.52	6.48	9.00	0.90	1.80	13.32	5.34
Monocytes[x]	1.44	2.40	0.96	1.60	1.80	1.68	2.16	1.88	0.60	0.90	1.48	0.99
Lymphocytes[x]	7.92	9.60	4.48	7.33	13.20	4.80	9.36	9.12	13.50	12.30	22.20	16.00

[x]Represents absolute values calculated from the total WBC.

TABLE 19

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 17 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	26.50	25.00	25.00	25.50	27.50	27.00	14.50	23.00	27.00	23.00	26.00	25.33
Hb. (gms. %)	7.60	7.60	6.80	7.33	7.80	7.20	3.60	6.20	6.80	6.80	6.40	6.66
Total WBC (count x 378)	15.00	24.00	15.00	18.00	9.00	21.00	15.00	15.00	32.00	18.00	10.00	20.00
PMN cells[x]	3.30	11.76	1.65	5.57	1.80	9.24	1.80	4.28	7.04	4.68	1.90	4.54
Monocytes[x]	1.20	3.36	2.10	2.20	1.44	2.73	0.75	1.64	14.72	1.80	1.50	6.01
Lymphocytes[x]	10.50	8.88	11.25	10.21	5.76	9.03	12.45	9.08	10.24	11.52	6.60	9.45

[x]Represents absolute values calculated from the total WBC.

TABLE 20

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 21 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	25.00	27.50	24.00	25.50	31.00	29.00	29.00	29.66	30.00	27.00	31.00	29.33
Hb. (gms. %)	7.20	8.20	7.20	7.53	9.00	8.60	8.60	8.73	8.20	7.80	8.60	8.20
Total WBC (count x 378)	11.00	15.00	16.00	14.00	19.00	20.00	22.00	20.33	14.00	22.00	26.00	20.66
PMN cells[x]	1.87	3.00	3.52	2.80	5.32	5.20	2.42	4.31	3.22	4.18	6.24	4.54
Monocytes[x]	0.33	0.90	0.96	0.73	1.33	0.80	0.88	1.00	0.70	0.88	2.08	1.22
Lymphocytes[x]	8.80	11.10	11.52	10.47	12.35	14.00	18.70	15.02	10.08	16.94	17.68	14.90

[x]Represents absolute values calculated from total WBC.

TABLE 21

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 28 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	25.50	28.50	29.00	27.66	25.50	31.50	27.50	28.16	28.00	31.00	32.00	30.33
Hb. (gms. %)	6.80	7.80	7.80	7.46	7.60	8.60	7.80	8.00	7.80	9.00	9.00	8.60
Total WBC (count x 378)	18.00	13.00	23.00	18.00	11.00	21.00	20.00	17.33	17.00	19.00	21.00	19.00
PMN cells^x	4.68	1.82	4.60	3.70	2.86	7.56	6.40	5.61	2.89	4.94	5.88	4.57
Monocytes^x	0.54	1.17	1.15	0.95	0.44	1.68	1.40	1.17	0.68	2.09	1.47	1.41
Lymphocytes^x	12.78	10.01	17.25	13.35	7.70	11.76	12.20	10.55	13.43	11.97	13.65	13.02

^{x}Represents absolute values calculated from total WBC.

TABLE 22

HEMATOLOGICAL OBSERVATIONS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 35 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	28.50	27.50	18.00	24.66	23.50	22.50	27.00	24.33	27.50	25.00	28.00	26.83
Hb. (gms. %)	8.60	8.20	5.40	7.40	6.80	6.40	7.80	7.00	8.20	7.60	8.60	8.13
Total WBC (count x 378)	13.00	16.00	16.00	15.00	19.00	24.00	30.00	24.33	16.00	20.00	16.00	17.33
PMN cells[x]	1.43	2.40	0.64	1.49	3.80	6.00	4.50	4.76	2.56	1.60	3.68	2.61
Monocytes[x]	0.78	0.48	0.96	0.74	2.66	1.20	5.10	2.99	1.28	2.00	1.44	1.57
Lymphocytes[x]	10.79	13.12	14.40	12.77	12.54	16.80	20.40	16.58	12.16	16.40	10.88	13.15

[x]Represents absolute values calculated from the total WBC.

TABLE 23

HEMATOLOGICAL OBSERVATIONS OF UNCHALLENGED AND CHALLENGED SERIES AT 37 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	27.50	28.50	26.00	27.33	24.00	25.00	27.50	25.50	29.00	25.00	23.00	25.66
Hb. (gms. %)	8.60	8.60	7.80	8.33	7.60	7.60	7.60	7.60	8.60	7.60	6.80	7.66
Total WBC (count x 378)	16.00	19.00	13.00	16.00	22.00	19.00	8.00	16.33	14.00	19.00	13.00	15.33
PMN cells^x	2.08	2.09	0.91	1.69	3.96	3.04	1.36	2.79	2.24	2.09	1.69	2.01
Monocytes^x	0.96	1.33	0.78	1.02	3.08	2.28	0.48	1.94	1.40	1.71	1.43	1.51
Lymphocytes^x	12.96	15.58	11.31	13.28	14.96	13.68	6.16	11.60	10.36	15.20	9.88	11.81

TABLE 23 (extended)

Treatment Series	Uninfected Challenged				5,000 Challenged				50,000 Challenged			
Bird Number	10	11	12	Mean	13	14	15	Mean	16	17	18	Mean
PCV (%)	26.00	27.00	29.00	27.33	24.00	25.00	27.00	24.33	26.00	26.00	27.00	26.33
Hb. (gms. %)	8.20	8.60	8.60	8.46	6.80	7.20	8.20	7.73	7.20	7.60	7.60	7.46
Total WBC (count x 378)	34.00	16.00	20.00	23.33	22.00	18.00	18.00	19.33	8.00	12.00	16.00	12.00
PMN cells[x]	5.78	2.88	2.40	3.69	3.30	3.42	0.72	2.48	1.60	1.56	1.44	1.53
Monocytes[x]	2.72	1.60	0.20	1.50	1.54	2.34	2.34	2.07	0.32	1.80	0.00	0.71
Lymphocytes[x]	25.50	11.52	17.40	18.14	17.16	12.24	14.94	14.78	6.08	8.64	14.56	9.76

[x]Represents absolute values calculated from the total WBC.

TABLE 24

HEMATOLOGICAL OBSERVATIONS OF UNCHALLENGED AND CHALLENGED SERIES AT 39 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	28.50	29.00	26.00	27.83	28.00	30.00	26.00	28.00	29.00	25.00	24.00	26.00
Hb. (gms. %)	8.60	9.00	8.20	8.60	8.20	9.00	7.80	8.33	8.60	7.60	7.20	7.80
Total WBC (count x 378)	12.00	13.00	16.00	13.66	13.00	19.00	13.00	15.00	13.00	22.00	8.00	14.33
PMN cells^x	1.44	1.43	2.56	1.81	2.60	2.85	2.34	2.60	2.21	2.20	1.60	2.00
Monocytes^x	0.84	0.78	1.28	0.96	1.30	1.52	1.43	1.41	1.04	1.32	0.72	1.03
Lymphocytes^x	9.72	10.79	12.16	10.89	9.10	14.63	9.23	10.99	9.75	18.48	5.68	11.30

TABLE 24 (extended)

Treatment Series	Uninfected Challenged				5,000 Challenged				50,000 Challenged			
Bird Number	10	11	12	Mean	13	14	15	Mean	16	17	18	Mean
PCV (%)	24.00	24.00	30.00	26.00	30.00	27.50	28.00	28.50	28.50	29.00	30.00	29.16
Hb. (gms. %)	7.20	7.60	8.20	7.66	9.00	7.80	8.20	8.66	8.20	9.40	9.40	9.00
Total WBC (count x 378)	10.00	11.00	12.00	11.00	19.00	24.00	13.00	18.66	19.00	19.00	14.00	17.33
PMN cells^x	0.90	1.69	7.14	3.24	0.76	7.68	1.69	3.38	3.80	2.85	5.04	3.90
Monocytes^x	0.30	0.65	0.00	0.32	0.76	0.72	0.52	0.66	0.19	0.19	0.98	0.45
Lymphocytes^x	13.80	10.66	13.86	12.77	17.48	15.60	10.79	14.62	15.01	15.96	7.98	12.98

^{x}Represents absolute values calculated from the total WBC.

TABLE 25

HEMATOLOGICAL OBSERVATIONS OF UNCHALLENGED AND CHALLENGED SERIES AT 42 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	26.00	26.00	27.50	26.50	24.00	25.00	23.00	24.00	28.00	28.00	29.00	28.33
Hb. (gms. %)	7.80	7.80	8.20	7.93	7.60	7.80	7.20	7.53	8.60	8.20	9.00	8.60
Total WBC (count x 378)	14.00	8.00	19.00	14.00	13.00	19.00	13.00	15.00	9.00	14.00	16.00	13.00
PMN cells^{x}	4.20	2.64	5.70	4.18	1.69	3.80	2.73	2.74	2.07	2.80	1.76	2.21
Monocytes^{x}	0.98	0.48	2.09	1.18	0.52	1.33	1.17	1.01	0.36	0.98	1.60	0.98
Lymphocytes^{x}	8.82	4.88	11.21	8.30	10.79	13.87	9.10	11.25	6.57	10.22	12.64	9.81

TABLE 25 (extended)

Treatment Series	Uninfected Challenged				5,000 Challenged				50,000 Challenged			
Bird Number	10	11	12	Mean	13	14	15	Mean	16	17	18	Mean
PCV (%)	20.00	19.00	20.00	19.66	26.50	24.00	25.00	25.16	26.50	28.50	26.50	27.16
Hb. (gms. %)	5.40	4.60	5.40	5.13	7.80	7.60	7.20	7.53	8.20	8.20	7.80	8.06
Total WBC (count x 378)	16.00	8.00	19.00	14.33	25.00	12.00	10.00	15.66	25.00	14.00	10.00	16.33
PMN cells[x]	4.16	1.76	5.32	3.75	4.00	2.64	2.20	2.95	5.00	3.08	3.70	3.93
Monocytes[x]	2.24	0.88	2.47	1.86	0.75	0.84	0.10	0.56	1.75	0.56	0.10	0.80
Lymphocytes[x]	9.60	5.36	11.21	8.72	20.25	8.52	7.70	12.15	18.25	10.36	6.20	11.60

[x]Represents absolute values calculated from the total WBC.

TABLE 26

HEMATOLOGICAL OBSERVATIONS OF UNCHALLENGED AND CHALLENGED SERIES AT 45 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
PCV (%)	27.00	25.00	26.00	26.00	30.00	24.00	23.00	25.66	31.00	25.00	27.50	27.83
Hb. (gms. %)	7.80	7.60	7.60	7.66	8.60	7.20	6.80	7.53	8.60	7.60	8.20	8.13
Total WBC (count x 378)	15.00	19.00	9.00	14.33	8.00	14.00	13.00	11.66	13.00	20.00	17.00	16.66
PMN cells^x	1.65	2.66	1.89	2.07	2.56	2.80	1.17	2.18	2.34	3.80	4.25	3.46
Monocytes^x	1.20	1.14	0.63	0.99	0.40	0.84	0.91	0.71	1.43	0.80	1.19	1.14
Lymphocytes^x	12.15	15.20	6.48	11.23	5.04	10.36	10.92	8.77	9.23	15.40	11.56	12.06

TABLE 26 (extended)

Treatment Series	Uninfected Challenged				5,000 Challenged				50,000 Challenged			
Bird Number	10	11	12	Mean	13	14	15	Mean	16	17	18	Mean
PCV (%)	21.00	20.00	23.00	21.33	28.00	26.00	27.00	27.00	30.00	30.00	32.00	30.66
Hb. (gms. %)	5.80	5.40	6.40	5.86	7.80	7.20	8.20	7.73	8.60	8.60	8.60	8.60
Total WBC (count x 378)	18.00	13.00	16.00	15.66	13.00	10.00	14.00	12.33	18.00	12.00	26.00	18.66
PMN cells^x	2.70	2.08	3.20	2.66	3.51	1.00	2.24	2.25	3.06	3.06	5.46	4.04
Monocytes^x	0.18	0.52	0.48	0.39	0.26	0.10	0.42	0.26	0.90	0.84	1.56	1.10
Lymphocytes^x	15.12	10.40	12.32	12.61	9.23	8.90	11.34	9.82	14.04	7.56	18.98	13.52

^{x}Represents absolute values calculated from the total WBC.

TABLE 27

STATISTICAL ANALYSIS ("t" VALUES) OF UNINFECTED CONTROL VERSUS 500 TREATMENT SERIES

Day Post-Treatment	PCV	Hemo-globin	Total WBC	PMN Cells	Mono-cytes	Lympho-cytes	"t" needed at 0.01 Level of Significance
3	-0.33	0.22	-0.67	-0.38	-0.55	-0.07	2.69
6	-0.17	-0.77	1.03	-0.24	-0.14	1.27	
9	1.47	0.68	-2.17	0.37	-0.40	-1.94	2.90
13	-0.35	-0.56	-5.06x	-0.35	-1.34	-3.40x	
17	0.36	0.45	-0.85	0.54	0.10	-1.18	
21	-1.53	-1.38	-0.23	-0.36	0.11	0.03	
28	-0.57	-1.97	1.02	-0.62	-0.66	1.62	
35	-0.81	-0.47	-.17	-2.18	0.26	1.69	3.03
37	0.36	-0.24	-1.54	-0.12	1.32	-1.78	
39	-0.90	-1.50	0.74	1.76	0.89	-1.10	
42	-0.54	-1.15	-0.80	-0.02	0.10	-0.70	
45	-3.32x	-2.19	-1.02	-0.49	0.19	-0.41	

xSignificant at P = 0.01.

TABLE 28

STATISTICAL ANALYSIS ("t" VALUES) OF UNINFECTED CONTROL VERSUS 500 + 5,000 TREATMENT SERIES

Day Post-Treatment	PCV	Hemo-globin	Total WBC	PMN Cells	Mono-cytes	Lympho-cytes	"t" needed at 0.01 Level of Significance
9	1.21	1.14	-1.20	0.04	-0.96	-0.64	2.90
13	0.69	0.80	-4.58x	-0.77	-0.34	-3.08x	
17	0.72	1.83	0.28	0.69	0.09	-0.33	
21	-1.71	-0.81	-0.29	0.34	-0.61	0.29	
28	0.27	0.10	-0.06	1.17	-1.24	-0.42	3.03
35	0.36	0.22	0.29	-1.57	0.22	1.34	
37	-0.18	-0.34	-1.37	-1.15	0.09	-0.32	
39	-2.25	-2.42	0.68	0.09	1.21	-0.01	
42	0.63	-0.23	0.40	1.62	0.40	-1.07	
45	-2.24	-1.85	0.29	0.05	0.39	0.03	

xSignificant at P = 0.01.

TABLE 29

STATISTICAL ANALYSIS ("t" VALUES) OF UNINFECTED CONTROL VERSUS 500 + 5,000 + 50,000 TREATMENT SERIES

Day Post-Treatment	PCV	Hemoglobin	Total WBC	PMN Cells	Monocytes	Lymphocytes	"t" needed at 0.01 Level of Significance
17	0.36	0.69	-0.51	-0.73	-0.23	-0.22	
21	-0.90	-1.73	-0.91	0.26	-1.00	-0.54	
28	0.54	0.00	-0.80	1.49	-0.56	-1.57	
35	-0.90	-1.62	-0.52	-2.11	-0.09	1.21	3.03
37	0.72	0.23	-1.37	1.36	-0.60	-1.93	
39	-0.36	0.00	1.42	0.13	-0.67	0.82	
42	1.08	1.73	-0.40	0.09	-0.44	-0.21	
45	-3.32[x]	-2.42	-0.40	0.18	0.33	-0.34	

[x]Significant at P = 0.01.

TABLE 30

STATISTICAL ANALYSIS ("t" VALUES) OF CHALLENGED UNINFECTED CONTROL VERSUS CHALLENGED VARIOUS TREATMENT SERIES

Day Post-Challenge	PCV	Hemoglobin	Total WBC	PMN Cells	Monocytes	Lymphocytes	"t" needed at 0.01 Level of Significance
control challenged versus 500 challenged							
2	0.70	0.21	-1.21	0.92	0.97	-2.18	
4	0.00	0.33	0.37	0.45	0.10	-0.07	3.03
7	-3.64x	-6.03x	-0.74	0.55	0.09	-0.17	
10	-3.72x	-7.11x	-0.37	-1.11	0.05	0.49	
control challenged versus 500 + 5,000 challenged							
2	-0.31	-0.87	0.58	1.29	1.07	-1.01	
4	0.39	-0.21	-1.58	-3.06x	-3.37x	2.63	3.03
7	-4.34x	-6.57x	-0.16	1.14	0.89	-1.43	
10	-3.48x	-7.11	-0.74	-0.59	-0.87	-0.62	
control challenged versus 500 + 5,000 + 50,000 challenged							
2	-0.23	-1.10	0.79	-0.15	1.98	0.22	
4	0.54	0.00	1.64	1.13	0.53	0.20	3.03
7	-5.04x	-7.11x	-0.21	0.29	0.41	0.60	
10	-5.00x	-8.31x	-0.05	-1.07	0.54	0.48	

xSignificant at P = 0.01.

TABLE 31

STATISTICAL ANALYSIS ("t" VALUES) OF UNCHALLENGED VERSUS CHALLENGED SERIES

Day Post-Challenge	PCV	Hemo-globin	Total WBC	PMN Cells	Mono-cytes	Lympho-cytes	"t" needed at 0.01 Level of Significance
			control unchallenged versus control challenged				
2	-0.78	-0.51	-3.16x	-2.56	-2.16	-0.22	2.65
4	-1.01	-0.73	-1.47	0.26	0.53	-1.71	
7	5.18x	6.23x	0.00	0.08	0.69	0.28	
10	2.41	5.70x	-0.16	0.35	-0.44	-0.17	
			500 unchallenged versus 500 challenged				
2	-0.39	0.00	-3.59x	-1.52	-2.24	-0.90	2.65
4	-0.23	0.94	-1.79	-0.96	-0.08	-0.77	
7	2.01	1.56	0.00	-0.44	-0.85	0.76	
10	1.55	0.94	0.42	-0.29	-0.24	0.69	
			500 + 5,000 unchallenged versus 500 + 5,000 challenged				
2	-0.93	0.94	-1.96	-0.17	-1.15	-0.94	2.65
4	1.31	1.25	-3.68x	-2.87x	-3.79x	0.92	
7	0.31	0.20	-0.53	-0.30	-0.11	-0.16	
10	0.85	0.62	-1.16	-0.29	0.12	-0.84	
			500 + 5,000 + 50,000 unchallenged versus 500 + 5,000 + 50,000 challenged				
2	-1.63	-1.67	-1.74	-3.98x	0.30	1.34	2.65
4	-0.15	-0.73	-1.16	1.27	0.53	-2.26	
7	-0.78	-2.07	0.16	0.29	0.08	-0.12	
10	0.31	0.00	0.16	-0.55	-0.16	0.62	

xSignificant at $P = 0.01$.

TABLE 32

STATISTICAL ANALYSIS ("t" VALUES) OF UNINFECTED CONTROL VERSUS 5,000 TREATMENT SERIES

Day Post-Treatment	PCV	Hemo-globin	Total WBC	PMN Cells	Mono-cytes	Lympho-cytes	"t" needed at 0.01 Level of Significance
3	-0.12	-0.25	-1.44	-1.56	-0.34	-1.07	
6	3.39x	2.80	-0.27	-0.60	-0.42	0.18	
9	0.56	0.74	-0.68	-0.13	0.36	-1.18	
13	0.00	0.17	-0.96	-1.21	-0.21	-0.60	
17	0.93	1.40	0.62	0.60	0.42	0.38	2.89
21	-1.54	-1.30	-0.70	-0.20	-1.52		
28	-0.19	0.67	0.14	-0.89	-0.16	0.94	
35	0.12	0.49	-1.92	-1.52	-1.68	-1.27	
37	0.68	0.90	-0.07	-0.51	-0.69	0.56	
39	0.06	0.33	-0.28	-0.37	-0.34	-0.03	
42	0.93	0.49	-0.21	0.67	0.13	-0.99	
45	0.13	0.16	0.55	-0.05	0.21	0.82	

xSignificant at P = 0.01.

TABLE 33

STATISTICAL ANALYSIS ("t" VALUES) OF UNINFECTED CONTROL VERSUS 50,000 TREATMENT SERIES

Day Post-Treatment	PCV	Hemo-globin	Total WBC	PMN Cells	Mono-cytes	Lympho-cytes	"t" needed at 0.01 Level of Significance
3	0.06	0.00	-1.37	-0.48	-0.17	-1.81	
6	5.56[x]	4.86[x]	-2.88	-1.94	-1.27	-2.71	
9	2.53	2.63	0.34	-0.67	1.65	0.30	
13	1.23	1.46	-1.44	0.49	0.45	-2.90[x]	
17	0.06	0.83	-0.41	0.48	-2.84	0.25	2.89
21	-1.42	-0.83	-1.37	-0.81	-0.37	-1.48	
28	-0.99	-1.41	-0.21	-0.40	-0.34	0.11	
35	-0.80	-0.90	-0.48	-0.52	-0.62	-0.13	
37	0.62	0.83	0.14	-0.15	-0.37	0.49	
39	0.68	0.99	0.14	-0.99	-0.05	-0.14	
42	-0.68	-0.83	0.21	0.92	0.15	-0.51	
45	-0.68	-0.59	-0.48	-0.65	-0.11	-0.28	

[x]Significant at P = 0.01.

TABLE 34

STATISTICAL ANALYSIS ("t" VALUES) OF UNCHALLENGED VERSUS CHALLENGED SERIES

Day Post-Challenge	PCV	Hemoglobin	Total WBC	PMN Cells	Monocytes	Lymphocytes	"t" needed at 0.01 Level of Significance
			control unchallenged versus control challenged				
2	0.00	-0.32	-1.78	-1.69	-0.99	-1.46	
4	1.22	2.20	-0.65	-1.21	1.31	-0.57	2.68
7	5.00[x]	7.80[x]	-0.08	0.32	-1.40	-0.13	
10	3.11[x]	4.21[x]	-0.32	-0.50	1.23	-0.41	
			5,000 unchallenged versus 5,000 challenged				
2	0.78	-0.32	-0.73	0.26	-0.27	0.96	
4	-0.33	-0.77	-0.89	0.66	1.54	-1.09	2.68
7	-0.77	0.00	-0.16	-0.18	0.92	-0.27	
10	-0.89	-0.47	-0.16	-0.06	0.92	-0.32	
			50,000 unchallenged versus 50,000 challenged				
2	-0.45	0.47	0.81	0.41	1.64	0.62	
4	-2.11	-2.81	-0.73	-1.61	1.19	-0.51	2.68
7	0.78	1.26	-0.81	-1.46	0.37	-0.54	
10	-1.88	-1.10	-0.49	-0.49	0.08	-0.44	

[x]Significant at P = 0.01.

TABLE 35

STATISTICAL ANALYSIS ("t" VALUES) OF CHALLENGED UNINFECTED CONTROL VERSUS CHALLENGED VARIOUS TREATMENT SERIES

Days Post-Challenge	PCV	Hemo-globin	Total WBC	PMN Cells	Mono-cytes	Lympho-cytes	"t" needed at 0.01 Level of Significance
			control challenged versus 5,000 challenged				
2	2.00	1.71	0.97	1.03	-1.17	1.01	2.93
4	-1.67	-2.35	-0.57	-0.12	-0.70	-0.56	
7	-3.67^x	-5.63^x	-0.32	0.68	2.68	-1.03	
10	-3.78^x	-4.39^x	0.81	0.35	0.27	0.84	
			control challenged versus 50,000 challenged				
2	0.67	2.35	2.75	1.83	1.63	2.51	2.93
4	-2.11	-3.15^x	-0.24	-0.56	-0.27	-0.06	
7	-5.00^x	-6.88^x	-0.48	-0.15	2.18	-0.86	
10	-6.22^x	-6.43^x	-0.73	-1.17	-1.46	-0.27	

^{x}Significant at P = 0.01.

TABLE 36

SERUM ANALYSIS OF UNINFECTED BIRDS AT 0 DAY

Treatment Series	Uninfected			
Bird Number	1	2	3	Mean
Total Protein (gms. %)	3.65	3.10	3.10	3.28
Albumin^x	1.72	1.55	1.58	1.62
Alpha-1 Globulin^x	0.18	0.12	0.12	0.14
Alpha-2 Globulin^x	0.29	0.22	0.19	0.23
Beta Globulin^x	0.69	0.56	0.50	0.58
Gamma Globulin^x	0.77	0.65	0.71	0.71

^{x}Represents absolute values calculated from the total protein.

TABLE 37

SERUM ANALYSIS OF UNINFECTED CONTROL AND 500 TREATMENT SERIES AT 3 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
Total Protein (gms. %)	3.10	3.35	3.65	3.37	3.35	3.10	3.10	3.18
Albumin^{x}	1.55	1.64	1.57	1.59	1.54	1.18	1.30	1.34
Alpha-1 Globulin^{x}	0.16	0.10	0.26	0.17	0.20	0.16	0.19	0.18
Alpha-2 Globulin^{x}	0.25	0.34	0.29	0.29	0.27	0.16	0.25	0.23
Beta Globulin^{x}	0.68	0.67	0.77	0.71	0.67	0.52	0.68	0.62
Gamma Globulin^{x}	0.46	0.60	0.76	0.61	0.67	1.08	0.68	0.81

^{x}Represents absolute values calculated from the total protein.

TABLE 38

SERUM ANALYSIS OF UNINFECTED CONTROL AND 500 TREATMENT SERIES AT 6 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
Total Protein (gms. %)	2.55	3.35	2.55	2.82	2.85	2.85	2.85	2.85
Albumin^x	1.22	1.54	1.28	1.35	1.34	1.17	1.20	1.24
Alpha-1 Globulin^x	0.08	0.17	0.10	0.12	0.14	0.20	0.23	0.19
Alpha-2 Globulin^x	0.18	0.17	0.20	0.18	0.23	0.20	0.20	0.21
Beta Globulin^x	0.51	0.77	0.46	0.58	0.51	0.66	0.63	0.60
Gamma Globulin^x	0.56	0.70	0.51	0.59	0.63	0.62	0.59	0.61

^{x}Represents absolute values calculated from the total protein.

TABLE 39

SERUM ANALYSIS OF UNINFECTED CONTROL, 500, AND 500 + 5,000 TREATMENT SERIES AT 9 DAYS

Treatment Series	Uninfected				500				500 + 5,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.10	2.55	2.85	2.83	3.10	2.85	3.10	3.02	3.65	3.10	2.85	3.20
Albumin[x]	1.40	1.20	1.23	1.27	1.43	1.51	1.61	1.52	1.35	1.36	1.34	1.35
Alpha-1 Globulin[x]	0.12	0.10	0.14	0.12	0.16	0.14	0.12	0.14	0.22	0.19	0.14	0.18
Alpha-2 Globulin[x]	0.28	0.20	0.26	0.25	0.22	0.17	0.22	0.20	0.18	0.22	0.23	0.21
Beta Globulin[x]	0.65	0.59	0.60	0.61	0.58	0.51	0.59	0.56	0.84	0.61	0.60	0.72
Gamma Globulin[x]	0.65	0.46	0.62	0.58	0.71	0.52	0.56	0.60	1.06	0.62	0.54	0.74

[x]Represents absolute values calculated from the total protein.

TABLE 40

SERUM ANALYSIS OF UNINFECTED CONTROL, 500, AND 500 + 5,000 TREATMENT SERIES AT 13 DAYS

Treatment Series	Uninfected				500				500 + 5,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.65	3.50	3.50	3.55	2.85	3.35	3.50	3.22	3.35	3.10	3.95	3.46
Albumin^x	1.68	1.58	1.43	1.56	1.43	1.34	1.30	1.35	1.47	1.30	1.78	1.52
Alpha-1 Globulin^x	0.11	0.18	0.21	0.17	0.14	0.17	0.14	0.15	0.13	0.12	0.12	0.12
Alpha-2 Globulin^x	0.37	0.24	0.35	0.32	0.23	0.33	0.28	0.28	0.34	0.28	0.36	0.32
Beta Globulin^x	0.73	0.70	0.74	0.72	0.48	0.74	0.70	0.64	0.70	0.71	0.98	0.80
Gamma Globulin^x	0.76	0.80	0.77	0.78	0.57	0.77	1.08	0.80	0.70	0.69	0.71	0.70

^{x}Represents absolute values calculated from the total protein.

TABLE 41

SERUM ANALYSIS OF UNINFECTED CONTROL, 500, 500 + 5,000, AND 500 + 5,000 + 50,000 TREATMENT SERIES AT 17 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
Total Protein (gms. %)	3.10	3.35	3.95	3.46	3.10	3.10	3.35	3.18
Albumin[x]	1.40	1.35	1.46	1.40	1.21	1.15	1.44	1.26
Alpha-1 Globulin[x]	0.12	0.13	0.24	0.16	0.16	0.18	0.20	0.18
Alpha-2 Globulin[x]	0.28	0.27	0.23	0.26	0.22	0.21	0.23	0.22
Beta Globulin[x]	0.65	0.73	1.15	0.84	0.86	0.78	0.81	0.81
Gamma Globulin[x]	0.65	0.87	0.87	0.80	0.65	0.78	0.67	0.71

TABLE 41 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
Total Protein (gms. %)	3.95	3.65	2.98	3.53	2.40	3.90	4.25	3.51
Albumin[x]	0.59	1.17	1.04	0.93	0.74	1.29	1.57	1.20
Alpha-1 Globulin[x]	0.20	0.22	0.21	0.21	0.14	0.27	0.21	0.21
Alpha-2 Globulin[x]	0.43	0.22	0.15	0.27	0.14	0.27	0.38	0.26
Beta Globulin[x]	1.19	0.99	0.75	0.98	0.46	0.94	1.06	0.82
Gamma Globulin[x]	1.54	1.05	0.83	1.14	0.91	1.13	1.02	1.02

[x]Represents absolute values calculated from the total protein.

TABLE 42

SERUM ANALYSIS OF UNINFECTED CONTROL, 500, 500 + 5,000, AND 500 + 5,000 + 50,000 TREATMENT SERIES AT 21 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
Total Protein (gms. %)	3.35	3.65	3.35	3.45	3.65	4.25	3.65	3.84
Albumin[x]	1.47	0.99	1.44	1.30	1.50	1.70	1.68	1.63
Alpha-1 Globulin[x]	0.20	0.15	0.20	0.19	0.26	0.25	0.22	0.24
Alpha-2 Globulin[x]	0.27	0.18	0.27	0.24	0.33	0.30	0.22	0.28
Beta Globulin[x]	0.80	1.13	0.84	0.92	0.94	1.23	1.02	1.06
Gamma Globulin[x]	0.60	1.20	0.60	0.80	0.62	0.77	0.51	0.63

TABLE 42 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
Total Protein (gms. %)	3.10	3.10	3.65	3.28	3.50	2.85	3.80	3.38
Albumin^{x}	1.05	1.27	1.50	1.27	1.47	0.94	1.14	1.18
Alpha-1 Globulin^{x}	0.28	0.22	0.18	0.23	0.14	0.23	0.27	0.21
Alpha-2 Globulin^{x}	0.16	0.19	0.22	0.19	0.25	0.14	0.23	0.21
Beta Globulin^{x}	0.87	0.80	0.95	0.87	0.63	0.63	1.06	0.77
Gamma Globulin^{x}	0.74	0.62	0.80	0.72	1.01	0.91	1.10	1.01

^{x}Represents absolute values calculated from the total protein.

TABLE 43

SERUM ANALYSIS OF UNINFECTED CONTROL, 500, 500 + 5,000, AND 500 + 5,000 + 50,000 TREATMENT SERIES AT 28 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
Total Protein (gms. %)	3.65	3.10	3.35	3.37	3.10	3.50	3.10	3.23
Albumin[x]	1.53	1.33	1.54	1.47	1.36	1.40	1.18	1.31
Alpha-1 Globulin[x]	0.15	0.22	0.13	0.17	0.12	0.21	0.25	0.19
Alpha-2 Globulin[x]	0.21	0.19	0.21	0.20	0.16	0.25	0.19	0.20
Beta Globulin[x]	0.99	0.84	0.90	0.91	0.74	0.91	0.86	0.84
Gamma Globulin[x]	0.77	0.52	0.57	0.62	0.71	0.73	0.62	0.69

TABLE 43 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
Total Protein (gms. %)	3.50	3.10	3.10	3.23	3.10	3.35	3.65	3.37
Albumin[x]	1.09	1.40	1.18	1.22	0.99	1.24	1.50	1.24
Alpha-1 Globulin[x]	0.35	0.16	0.28	0.26	0.22	0.20	0.22	0.21
Alpha-2 Globulin[x]	0.17	0.19	0.16	0.17	0.12	0.17	0.22	0.17
Beta Globulin[x]	1.12	0.86	0.86	0.95	0.93	0.97	1.02	0.97
Gamma Globulin[x]	0.77	0.49	0.62	0.63	0.84	0.77	0.69	0.77

[x]Represents absolute values calculated from the total protein.

TABLE 44

SERUM ANALYSIS OF UNINFECTED CONTROL, 500, 500 + 5,000, AND 500 + 5,000 + 50,000 TREATMENT SERIES AT 35 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
Total Protein (gms. %)	3.35	4.25	3.65	3.75	3.65	3.50	3.65	3.60
Albumin^x	1.27	1.40	1.06	1.24	1.72	1.33	1.35	1.47
Alpha-1 Globulin^x	0.27	0.17	0.29	0.24	0.18	0.24	0.37	0.26
Alpha-2 Globulin^x	0.20	0.30	0.22	0.24	0.26	0.25	0.18	0.23
Beta Globulin^x	0.77	0.81	0.73	0.77	0.87	0.84	1.06	0.92
Gamma Globulin^x	0.84	1.57	1.35	1.25	0.62	0.84	0.69	0.72

TABLE 44 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
Total Protein (gms. %)	3.65	4.25	3.95	3.95	3.65	3.35	3.10	3.37
Albumin[x]	1.06	1.40	1.30	1.25	1.50	1.44	1.27	1.40
Alpha-1 Globulin[x]	0.40	0.17	0.16	0.24	0.22	0.13	0.18	0.18
Alpha-2 Globulin[x]	0.18	0.21	0.20	0.20	0.29	0.30	0.25	0.28
Beta Globulin[x]	1.02	1.06	1.19	1.09	0.88	0.80	0.78	0.82
Gamma Globulin[x]	0.99	1.40	1.10	1.16	0.76	0.67	0.62	0.68

[x]Represents absolute values calculated from the total protein.

TABLE 45

SERUM ANALYSIS OF VARIOUS UNCHALLENGED AND CHALLENGED SERIES AT 37 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
Total Protein (gms. %)	3.10	2.55	3.65	3.10	3.65	2.85	3.35	3.28
Albumin^x	1.33	1.12	1.28	1.24	1.42	0.97	1.37	1.25
Alpha-1 Globulin^x	0.16	0.13	0.29	0.19	0.22	0.22	0.24	0.23
Alpha-2 Globulin^x	0.28	0.18	0.26	0.24	0.22	0.14	0.30	0.22
Beta Globulin^x	0.71	0.64	0.99	0.78	0.95	0.77	0.74	0.82
Gamma Globulin^x	0.62	0.48	0.83	0.64	0.84	0.74	0.70	0.76

TABLE 45 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
Total Protein (gms. %)	3.80	4.25	4.40	4.15	3.80	4.25	2.98	3.68
Albumin^{x}	1.18	1.10	1.32	1.20	1.25	1.45	1.28	1.33
Alpha-1 Globulin^{x}	0.34	0.47	0.31	0.37	0.34	0.43	0.18	0.32
Alpha-2 Globulin^{x}	0.15	0.21	0.22	0.19	0.27	0.29	0.18	0.25
Beta Globulin^{x}	1.22	1.28	0.97	1.16	0.99	1.15	0.72	0.95
Gamma Globulin^{x}	0.91	1.19	1.58	1.23	0.95	0.93	0.62	0.83

TABLE 45 (extended)

Treatment Series	Uninfected Challenged				500 Challenged			
Bird Number	13	14	15	Mean	16	17	18	Mean
Total Protein (gms. %)	3.23	3.50	3.95	3.56	4.55	3.65	3.65	3.95
Albumin^x	1.32	1.37	1.46	1.38	1.46	1.31	1.28	1.35
Alpha-1 Globulin^x	0.23	0.28	0.24	0.25	0.27	0.29	0.18	0.25
Alpha-2 Globulin^x	0.19	0.21	0.27	0.22	0.23	0.22	0.26	0.24
Beta Globulin^x	0.74	0.94	0.99	0.89	1.23	0.91	1.13	1.09
Gamma Globulin^x	0.74	0.70	0.99	0.81	1.36	0.91	0.80	1.02

TABLE 45 (extended)

Treatment Series	500 + 5,000 Challenged				500 + 5,000 + 50,000 Challenged			
Bird Number	19	20	21	Mean	22	23	24	Mean
Total Protein (gms. %)	3.95	3.95	4.25	4.05	3.65	3.35	3.95	3.65
Albumin[x]	1.26	1.30	1.36	1.31	1.17	1.04	1.22	1.14
Alpha-1 Globulin[x]	0.28	0.20	0.30	0.26	0.26	0.20	0.28	0.25
Alpha-2 Globulin[x]	0.28	0.24	0.25	0.26	0.22	0.20	0.24	0.22
Beta Globulin[x]	0.91	1.14	1.02	1.02	0.98	0.77	1.19	0.98
Gamma Globulin[x]	1.22	1.07	1.32	1.20	1.02	1.14	1.02	1.06

[x]Represents absolute values calculated from the total protein.

TABLE 46

SERUM ANALYSIS OF VARIOUS UNCHALLENGED AND CHALLENGED SERIES AT 39 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
Total Protein (gms. %)	4.55	4.55	5.35	4.82	4.25	5.10	5.35	4.90
Albumin^x	1.14	1.64	1.45	1.41	1.70	1.48	1.55	1.58
Alpha-1 Globulin^x	0.50	0.36	0.37	0.41	0.21	0.51	0.37	0.36
Alpha-2 Globulin^x	0.27	0.23	0.37	0.29	0.30	0.26	0.37	0.31
Beta Globulin^x	1.18	0.96	1.34	1.16	0.98	1.38	1.55	1.30
Gamma Globulin^x	1.46	1.36	1.82	1.55	1.06	1.47	1.50	1.34

TABLE 46 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
Total Protein (gms. %)	4.80	4.55	4.25	4.53	4.25	4.55	4.25	4.35
Albumin^{x}	1.82	1.59	1.32	1.58	1.57	1.77	1.57	1.64
Alpha-1 Globulin^{x}	0.38	0.23	0.34	0.32	0.34	0.36	0.21	0.30
Alpha-2 Globulin^{x}	0.24	0.27	0.21	0.24	0.26	0.32	0.21	0.26
Beta Globulin^{x}	1.25	0.86	1.02	1.04	1.11	1.23	0.89	1.08
Gamma Globulin^{x}	1.10	1.59	1.36	1.35	0.97	0.87	1.36	1.07

TABLE 46 (extended)

Treatment Series	Uninfected Challenged				500 Challenged			
Bird Number	13	14	15	Mean	16	17	18	Mean
Total Protein (gms. %)	2.85	3.65	3.65	3.38	3.65	3.35	2.70	3.23
Albumin^x	0.97	1.39	1.39	1.25	1.35	1.27	1.12	1.25
Alpha-1 Globulin^x	0.26	0.29	0.29	0.28	0.37	0.20	0.15	0.24
Alpha-2 Globulin^x	0.14	0.26	0.22	0.21	0.22	0.17	0.30	0.23
Beta Globulin^x	0.74	0.91	0.91	0.85	0.91	0.67	0.64	0.74
Gamma Globulin^x	0.74	0.80	0.84	0.79	0.80	1.04	0.49	0.77

TABLE 46 (extended)

Treatment Series	500 + 5,000 Challenged				500 + 5,000 + 50,000 Challenged			
Bird Number	19	20	21	Mean	22	23	24	Mean
Total Protein (gms. %)	3.95	3.65	4.25	3.95	3.95	3.95	3.95	3.95
Albumin[x]	1.58	1.28	1.45	1.44	1.57	1.49	1.39	1.48
Alpha-1 Globulin[x]	0.28	0.44	0.55	0.42	0.45	0.32	0.40	0.39
Alpha-2 Globulin[x]	0.24	0.15	0.30	0.23	0.29	0.32	0.24	0.28
Beta Globulin[x]	1.03	0.98	1.23	1.08	0.84	0.90	0.90	0.88
Gamma Globulin[x]	0.82	0.80	0.72	0.78	0.80	0.92	1.02	0.91

[x]Represents absolute values calculated from the total protein.

TABLE 47

SERUM ANALYSIS OF VARIOUS UNCHALLENGED AND CHALLENGED SERIES AT 42 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
Total Protein (gms. %)	4.25	6.05	3.95	4.75	4.25	4.25	3.95	4.15
Albumin^{x}	1.15	1.88	1.66	1.56	1.83	1.23	1.62	1.56
Alpha-1 Globulin^{x}	0.30	0.61	0.67	0.53	0.26	0.26	0.28	0.27
Alpha-2 Globulin^{x}	0.26	0.24	0.24	0.25	0.26	0.17	0.24	0.22
Beta Globulin^{x}	0.97	1.63	0.40	1.00	0.97	1.23	0.98	1.06
Gamma Globulin^{x}	1.57	1.69	0.98	1.41	0.93	1.36	0.83	1.04

TABLE 47 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
Total Protein (gms. %)	3.23	3.80	4.40	3.81	4.25	3.95	3.65	3.95
Albumin[x]	1.52	1.60	1.67	1.60	1.83	1.54	1.20	1.52
Alpha-1 Globulin[x]	0.19	0.19	0.35	0.24	0.34	0.36	0.33	0.34
Alpha-2 Globulin[x]	0.26	0.30	0.31	0.29	0.38	0.32	0.26	0.32
Beta Globulin[x]	0.74	0.91	1.23	0.96	0.89	0.94	0.95	0.93
Gamma Globulin[x]	0.52	0.80	0.84	0.72	0.81	0.79	0.91	0.84

TABLE 47 (extended)

Treatment Series	Uninfected Challenged				500 Challenged			
Bird Number	13	14	15	Mean	16	17	18	Mean
Total Protein (gms. %)	3.10	3.50	4.10	3.57	3.35	3.65	3.80	3.60
Albumin[x]	0.99	1.44	1.56	1.33	1.47	1.28	1.41	1.39
Alpha-1 Globulin[x]	0.12	0.18	0.21	0.17	0.20	0.18	0.11	0.16
Alpha-2 Globulin[x]	0.25	0.32	0.37	0.31	0.24	0.29	0.30	0.28
Beta Globulin[x]	0.87	0.88	0.90	0.88	0.67	0.99	0.95	0.87
Gamma Globulin[x]	0.87	0.70	1.06	0.88	0.77	0.91	1.03	0.90

TABLE 47 (extended)

Treatment Series	500 + 5,000 Challenged				500 + 5,000 + 50,000 Challenged			
Bird Number	19	20	21	Mean	22	23	24	Mean
Total Protein (gms. %)	3.80	4.10	3.95	3.95	3.50	3.95	3.95	3.80
Albumin[x]	1.18	1.23	1.34	1.25	1.44	1.58	0.83	1.28
Alpha-1 Globulin[x]	0.15	0.49	0.36	0.33	0.24	0.20	0.36	0.27
Alpha-2 Globulin[x]	0.27	0.33	0.16	0.25	0.21	0.24	0.31	0.25
Beta Globulin[x]	0.80	1.11	0.95	0.95	0.84	0.87	1.11	0.94
Gamma Globulin[x]	1.40	0.94	1.14	1.16	0.77	1.06	1.34	1.06

[x]Represents absolute values calculated from the total protein.

TABLE 48

SERUM ANALYSIS OF VARIOUS UNCHALLENGED AND CHALLENGED SERIES AT 45 DAYS

Treatment Series	Uninfected				500			
Bird Number	1	2	3	Mean	4	5	6	Mean
Total Protein (gms. %)	2.55	3.80	4.55	3.63	3.65	3.65	4.25	3.85
Albumin^{x}	1.15	1.60	1.46	1.40	1.46	1.06	1.53	1.35
Alpha-1 Globulin^{x}	0.15	0.23	0.41	0.26	0.15	0.15	0.17	0.16
Alpha-2 Globulin^{x}	0.18	0.19	0.22	0.20	0.22	0.18	0.26	0.22
Beta Globulin^{x}	0.46	0.72	1.23	0.80	0.51	0.88	0.89	0.76
Gamma Globulin^{x}	0.61	1.06	1.23	0.97	1.31	1.38	1.40	1.36

TABLE 48 (extended)

Treatment Series	500 + 5,000				500 + 5,000 + 50,000			
Bird Number	7	8	9	Mean	10	11	12	Mean
Total Protein (gms. %)	3.65	3.95	4.25	3.95	3.65	3.35	3.23	3.41
Albumin^x	1.57	1.74	1.45	1.59	1.50	1.24	1.55	1.43
Alpha-1 Globulin^x	0.15	0.20	0.17	0.17	0.22	0.20	0.16	0.19
Alpha-2 Globulin^x	0.22	0.24	0.25	0.24	0.29	0.20	0.29	0.26
Beta Globulin^x	0.73	0.95	1.02	0.90	0.73	0.70	0.68	0.70
Gamma Globulin^x	0.98	0.82	1.36	1.05	0.91	1.01	0.55	0.82

TABLE 48 (extended)

Treatment Series	Uninfected Challenged				500 Challenged			
Bird Number	13	14	15	Mean	16	17	18	Mean
Total Protein (gms. %)	3.35	3.35	3.95	3.55	4.25	3.50	4.80	4.18
Albumin[x]	1.07	0.91	1.50	1.16	2.00	1.61	1.30	1.64
Alpha-1 Globulin[x]	0.37	0.18	0.16	0.24	0.13	0.11	0.28	0.17
Alpha-2 Globulin[x]	0.20	0.25	0.24	0.23	0.21	0.18	0.24	0.21
Beta Globulin[x]	0.77	1.13	0.87	0.92	0.94	0.59	1.49	1.00
Gamma Globulin[x]	0.94	0.88	1.18	1.00	0.97	1.01	1.49	1.16

TABLE 48 (extended)

Treatment Series	500 + 5,000 Challenged				500 + 5,000 + 50,000 Challenged			
Bird Number	19	20	21	Mean	22	23	24	Mean
Total Protein (gms. %)	3.95	3.35	3.35	3.55	3.35	4.10	3.65	3.70
Albumin[x]	1.98	1.51	0.80	1.43	1.54	1.64	1.61	1.60
Alpha-1 Globulin[x]	0.16	0.17	0.10	0.14	0.13	0.12	0.17	0.14
Alpha-2 Globulin[x]	0.24	0.13	0.17	0.18	0.17	0.16	0.23	0.19
Beta Globulin[x]	0.86	0.70	0.77	0.78	0.67	0.86	0.63	0.72
Gamma Globulin[x]	0.71	0.84	1.51	1.02	0.84	1.31	1.01	1.05

[x]Represents absolute values calculated from the total protein.

TABLE 49

SERUM ANALYSIS OF UNINFECTED BIRDS AT 0 DAY

Treatment Series	Uninfected			
Bird Number	1	2	3	Mean
Total Protein (gms. %)	3.65	3.35	3.95	3.65
Albumin[x]	1.79	1.71	1.81	1.77
Alpha-1 Globulin[x]	0.29	0.23	0.16	0.23
Alpha-2 Globulin[x]	0.22	0.17	0.32	0.23
Beta Globulin[x]	0.73	0.60	0.91	0.75
Gamma Globulin[x]	0.62	0.64	0.75	0.67

[x]Represents absolute values calculated from the total protein.

TABLE 50

SERUM ANALYSIS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 3 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.50	3.35	3.95	3.60	3.35	3.95	3.10	3.46	2.55	2.85	2.25	2.55
Albumin[x]	1.75	1.78	1.86	1.80	1.74	1.77	1.30	1.60	1.28	0.94	0.90	1.04
Alpha-1 Globulin[x]	0.25	0.17	0.12	0.18	0.13	0.20	0.19	0.17	0.10	0.29	0.16	0.18
Alpha-2 Globulin[x]	0.21	0.17	0.32	0.23	0.24	0.20	0.19	0.21	0.20	0.17	0.14	0.17
Beta Globulin[x]	0.73	0.63	0.94	0.77	0.64	0.95	0.80	0.80	0.46	0.71	0.42	0.53
Gamma Globulin[x]	0.56	0.60	0.71	0.62	0.60	0.83	0.62	0.68	0.51	0.74	0.63	0.63

[x]Represents absolute values calculated from the total protein.

TABLE 51

SERUM ANALYSIS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 6 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.10	3.10	2.85	3.02	2.85	1.70	2.55	2.37	1.70	2.00	2.85	2.18
Albumin[x]	1.46	1.46	1.11	1.34	1.40	0.77	1.17	1.11	0.73	1.02	0.83	0.86
Alpha-1 Globulin[x]	0.18	0.09	0.23	0.17	0.17	0.08	0.13	0.13	0.05	0.12	0.17	0.11
Alpha-2 Globulin[x]	0.22	0.18	0.26	0.22	0.17	0.10	0.20	0.16	0.20	0.14	0.17	0.16
Beta Globulin[x]	0.56	0.59	0.71	0.62	0.60	0.26	0.49	0.45	0.27	0.26	0.94	0.49
Gamma Globulin[x]	0.68	0.78	0.54	0.67	0.51	0.49	0.56	0.52	0.44	0.46	0.74	0.56

[x]Represents absolute values calculated from the total protein.

TABLE 52

SERUM ANALYSIS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 9 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.35	2.55	3.35	3.08	3.35	3.10	3.65	3.36	3.23	3.35	2.25	2.94
Albumin[x]	1.34	1.35	1.07	1.25	1.58	1.45	1.90	1.64	1.26	1.24	0.68	1.06
Alpha-1 Globulin[x]	0.30	0.10	0.24	0.21	0.13	0.16	0.22	0.17	0.26	0.30	0.38	0.31
Alpha-2 Globulin[x]	0.23	0.15	0.27	0.22	0.20	0.16	0.22	0.19	0.23	0.20	0.16	0.20
Beta Globulin[x]	0.74	0.49	0.70	0.64	0.70	0.62	0.80	0.71	0.61	0.67	0.49	0.59
Gamma Globulin[x]	0.74	0.46	1.07	0.76	0.74	0.71	0.51	0.65	0.87	0.94	0.54	0.78

[x]Represents absolute values calculated from the total protein.

TABLE 53

SERUM ANALYSIS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 13 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.23	2.85	3.35	3.14	3.65	3.50	3.35	3.50	3.50	3.23	2.85	3.19
Albumin[x]	1.65	1.51	1.78	1.65	1.75	1.96	1.41	1.71	1.02	1.36	1.20	1.19
Alpha-1 Globulin[x]	0.19	0.14	0.10	0.14	0.18	0.14	0.13	0.15	0.10	0.23	0.20	0.18
Alpha-2 Globulin[x]	0.23	0.20	0.20	0.21	0.29	0.21	0.20	0.23	0.49	0.19	0.17	0.28
Beta Globulin[x]	0.68	0.46	0.60	0.58	0.66	0.49	0.57	0.57	1.05	0.74	0.60	0.80
Gamma Globulin[x]	0.48	0.54	0.67	0.56	0.77	0.70	1.04	0.84	0.84	0.71	0.68	0.74

[x]Represents absolute values calculated from the total protein.

TABLE 54

SERUM ANALYSIS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 17 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.10	3.80	3.65	3.52	3.35	3.65	3.95	3.65	3.35	3.95	3.65	3.65
Albumin^x	1.40	1.60	1.68	1.56	1.64	1.50	1.66	1.60	0.97	1.58	1.68	1.41
Alpha-1 Globulin^x	0.09	0.38	0.18	0.22	0.07	0.26	0.20	0.18	0.40	0.20	0.15	0.25
Alpha-2 Globulin^x	0.25	0.23	0.37	0.28	0.30	0.26	0.24	0.27	0.27	0.20	0.33	0.27
Beta Globulin^x	0.59	0.91	0.69	0.73	0.64	0.83	0.91	0.79	0.84	0.67	0.69	0.73
Gamma Globulin^x	0.77	0.68	0.73	0.73	0.70	0.80	0.94	0.81	0.87	1.30	0.80	0.99

^{x}Represents absolute values calculated from the total protein.

TABLE 55

SERUM ANALYSIS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 21 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.65	3.23	3.65	3.51	2.85	3.35	3.65	3.28	3.23	3.35	3.65	3.41
Albumin[x]	1.68	1.68	1.86	1.74	1.25	1.57	1.57	1.46	1.55	1.31	1.57	1.48
Alpha-1 Globulin[x]	0.15	0.06	0.15	0.12	0.14	0.17	0.18	0.16	0.13	0.17	0.18	0.16
Alpha-2 Globulin[x]	0.18	0.29	0.22	0.23	0.23	0.27	0.22	0.24	0.16	0.17	0.26	0.20
Beta Globulin[x]	0.66	0.52	0.66	0.61	0.60	0.64	0.80	0.68	0.58	0.63	0.58	0.59
Gamma Globulin[x]	0.98	0.68	0.76	0.81	0.63	0.70	0.88	0.74	0.81	1.07	1.06	0.98

[x]Represents absolute values calculated from the total protein.

TABLE 56

SERUM ANALYSIS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 28 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.10	3.95	3.95	3.67	3.35	3.65	3.35	3.45	3.23	3.35	3.65	3.41
Albumin[x]	1.46	1.66	1.58	1.57	1.57	1.68	1.51	1.59	1.55	1.41	1.68	1.55
Alpha-1 Globulin[x]	0.09	0.24	0.24	0.19	0.13	0.15	0.13	0.14	0.13	0.17	0.26	0.19
Alpha-2 Globulin[x]	0.22	0.20	0.20	0.21	0.23	0.26	0.20	0.23	0.26	0.23	0.29	0.26
Beta Globulin[x]	0.59	0.83	0.99	0.80	0.67	0.65	0.47	0.60	0.55	0.60	0.69	0.61
Gamma Globulin[x]	0.74	1.02	0.94	0.90	0.74	0.91	1.04	0.89	0.74	0.94	0.73	0.80

[x]Represents absolute values calculated from the total protein.

TABLE 57

SERUM ANALYSIS OF UNINFECTED CONTROL, 5,000, AND 50,000 TREATMENT SERIES AT 35 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.35	3.23	3.65	3.41	3.35	2.98	4.25	3.53	3.23	3.10	3.65	3.32
Albumin^{x}	1.94	1.65	0.99	1.53	1.61	1.52	1.28	1.47	1.55	1.43	1.72	1.57
Alpha-1 Globulin^{x}	0.03	0.13	0.33	0.16	0.17	0.15	0.38	0.23	0.10	0.06	0.15	0.10
Alpha-2 Globulin^{x}	0.20	0.15	0.18	0.18	0.13	0.21	0.17	0.17	0.29	0.28	0.22	0.26
Beta Globulin^{x}	0.54	0.65	0.80	0.66	0.80	0.51	1.23	0.85	0.58	0.53	0.76	0.62
Gamma Globulin^{x}	0.64	0.65	1.35	0.88	0.64	0.59	1.19	0.81	0.71	0.80	0.80	0.77

^{x}Represents absolute values calculated from the total protein.

TABLE 58

SERUM ANALYSIS OF UNCHALLENGED AND CHALLENGED SERIES AT 37 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	4.25	3.35	2.85	3.48	3.35	3.65	3.10	3.37	3.95	3.65	3.10	3.57
Albumin[x]	1.53	1.44	1.23	1.40	0.87	1.10	0.99	0.99	1.34	1.24	1.36	1.31
Alpha-1 Globulin[x]	0.30	0.17	0.17	0.21	0.34	0.22	0.25	0.27	0.40	0.29	0.16	0.28
Alpha-2 Globulin[x]	0.26	0.30	0.17	0.24	0.20	0.22	0.12	0.18	0.24	0.26	0.18	0.23
Beta Globulin[x]	1.19	0.80	0.68	0.89	1.07	0.84	0.96	0.96	1.11	0.95	0.78	0.95
Gamma Globulin[x]	0.97	0.64	0.60	0.74	0.87	1.27	0.78	0.97	0.86	0.91	0.62	0.80

TABLE 58 (extended)

Treatment Series	Uninfected Challenged				5,000 Challenged				50,000 Challenged			
Bird Number	10	11	12	Mean	13	14	15	Mean	16	17	18	Mean
Total Protein (gms. %)	3.95	3.35	3.65	3.65	3.95	3.65	3.35	3.65	3.35	3.35	3.65	3.45
Albumin^x	1.50	1.34	1.39	1.41	1.26	1.20	1.07	1.18	1.04	1.04	1.17	1.08
Alpha-1 Globulin^x	0.20	0.20	0.29	0.23	0.28	0.22	0.24	0.25	0.20	0.24	0.22	0.22
Alpha-2 Globulin^x	0.32	0.24	0.26	0.27	0.24	0.26	0.20	0.23	0.24	0.20	0.26	0.23
Beta Globulin^x	0.95	0.80	0.95	0.90	1.19	1.10	1.07	1.12	0.77	0.80	0.98	0.85
Gamma Globulin^x	0.98	0.77	0.76	0.84	0.98	0.87	0.77	0.87	1.10	1.11	1.02	1.07

^{x}Represents absolute values calculated from the total protein.

TABLE 59

SERUM ANALYSIS OF UNCHALLENGED AND CHALLENGED SERIES AT 39 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	4.55	4.80	4.25	4.53	3.65	3.35	4.25	3.75	3.65	3.35	3.95	3.65
Albumin^x	1.18	1.30	1.28	1.25	1.28	1.07	1.57	1.31	1.35	1.24	1.58	1.39
Alpha-1 Globulin^x	0.46	0.29	0.21	0.32	0.18	0.24	0.30	0.24	0.18	0.30	0.28	0.25
Alpha-2 Globulin^x	0.27	0.38	0.26	0.30	0.22	0.17	0.30	0.23	0.18	0.17	0.31	0.22
Beta Globulin^x	1.14	1.25	1.06	1.15	0.73	0.84	1.11	0.89	0.73	0.91	0.75	0.80
Gamma Globulin^x	1.50	1.58	1.44	1.51	1.24	1.03	0.97	1.08	1.21	0.73	1.03	0.99

TABLE 59 (extended)

Treatment Series	Uninfected Challenged				5,000 Challenged				50,000 Challenged			
Bird Number	10	11	12	Mean	13	14	15	Mean	16	17	18	Mean
Total Protein (gms. %)	2.55	3.35	3.10	3.00	3.35	3.65	3.80	3.60	3.55	3.65	4.10	3.76
Albumin^x	1.02	1.27	1.18	1.16	1.34	0.69	1.10	1.04	1.41	0.95	0.91	1.09
Alpha-1 Globulin^x	0.03	0.27	0.22	0.17	0.24	0.33	0.29	0.29	0.17	0.33	0.40	0.30
Alpha-2 Globulin^x	0.18	0.20	0.25	0.21	0.20	0.25	0.33	0.26	0.23	0.33	0.26	0.27
Beta Globulin^x	0.66	0.80	0.80	0.75	0.87	1.02	0.63	0.84	0.97	0.80	0.97	0.91
Gamma Globulin^x	0.66	0.80	0.65	0.70	0.70	1.31	1.50	1.17	0.77	1.24	1.56	1.19

^{x}Represents absolute values calculated from the total protein.

TABLE 60

SERUM ANALYSIS OF UNCHALLENGED AND CHALLENGED SERIES AT 42 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	4.25	4.25	4.25	4.25	3.35	3.95	3.65	3.65	3.95	3.65	3.10	3.57
Albumin[x]	1.46	1.65	1.23	1.45	1.24	1.50	1.50	1.41	1.74	1.46	1.02	1.41
Alpha-1 Globulin[x]	0.58	0.30	0.60	0.49	0.17	0.28	0.26	0.24	0.28	0.29	0.25	0.27
Alpha-2 Globulin[x]	0.24	0.30	0.21	0.25	0.27	0.32	0.26	0.28	0.35	0.29	0.25	0.30
Beta Globulin[x]	0.90	0.94	1.27	1.04	0.80	0.99	0.87	0.89	0.79	0.84	0.81	0.81
Gamma Globulin[x]	1.07	1.06	0.94	1.02	0.87	0.86	0.76	0.83	0.79	0.77	0.77	0.78

TABLE 60 (extended)

Treatment Series	Uninfected Challenged				5,000 Challenged				50,000 Challenged			
Bird Number	10	11	12	Mean	13	14	15	Mean	16	17	18	Mean
Total Protein (gms. %)	3.10	3.10	3.10	3.10	3.95	3.65	3.65	3.75	3.65	3.65	3.35	3.55
Albumin^x	1.24	0.96	1.21	1.14	1.38	1.28	1.10	1.25	0.80	1.43	1.07	1.10
Alpha-1 Globulin^x	0.19	0.16	0.12	0.16	0.32	0.26	0.18	0.25	0.27	0.22	0.20	0.23
Alpha-2 Globulin^x	0.25	0.22	0.25	0.24	0.20	0.22	0.22	0.21	0.28	0.22	0.17	0.22
Beta Globulin^x	0.80	0.90	0.71	0.80	0.90	0.87	0.80	0.86	1.02	0.84	0.84	0.90
Gamma Globulin^x	0.62	0.86	0.81	0.76	1.15	1.02	1.35	1.17	1.28	0.94	1.07	1.10

^{x}Represents absolute values calculated from the total protein.

TABLE 61

SERUM ANALYSIS OF UNCHALLENGED AND CHALLENGED SERIES AT 45 DAYS

Treatment Series	Uninfected				5,000				50,000			
Bird Number	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
Total Protein (gms. %)	3.95	3.35	3.35	3.55	3.65	3.95	3.23	3.61	3.65	3.10	3.35	3.37
Albumin^x	1.54	1.37	1.36	1.42	1.61	1.34	1.49	1.48	1.46	1.52	1.24	1.41
Alpha-1 Globulin^x	0.20	0.17	0.47	0.28	0.15	0.20	0.16	0.17	0.26	0.12	0.20	0.19
Alpha-2 Globulin^x	0.20	0.20	0.22	0.21	0.18	0.24	0.19	0.20	0.29	0.25	0.17	0.24
Beta Globulin^x	0.82	0.64	0.53	0.66	0.77	0.91	0.74	0.81	0.73	0.65	0.77	0.72
Gamma Globulin^x	1.19	0.97	0.77	0.98	0.94	1.26	0.65	0.95	0.91	0.56	0.97	0.81

TABLE 61 (extended)

Treatment Series	Uninfected Challenged				5,000 Challenged				50,000 Challenged			
Bird Number	10	11	12	Mean	13	14	15	Mean	16	17	18	Mean
Total Protein (gms. %)	3.65	3.10	3.35	3.36	3.65	3.35	3.95	3.65	3.35	3.55	3.65	3.52
Albumin^x	1.42	0.80	1.10	1.10	1.86	1.54	1.58	1.66	1.50	1.37	1.58	1.48
Alpha-1 Globulin^x	0.18	0.20	0.34	0.24	0.15	0.20	0.20	0.18	0.17	0.20	0.20	0.19
Alpha-2 Globulin^x	0.18	0.22	0.24	0.21	0.22	0.13	0.24	0.20	0.17	0.20	0.23	0.20
Beta Globulin^x	0.77	1.01	0.80	0.86	0.77	0.67	0.83	0.76	0.70	0.67	0.57	0.65
Gamma Globulin^x	1.10	0.87	0.87	0.95	0.65	0.80	1.10	0.85	0.80	1.11	0.97	0.96

^{x}Represents absolute values calculated from the total protein.

TABLE 62

STATISTICAL ANALYSIS ("t" VALUES) OF UNINFECTED CONTROL VERSUS 500 TREATMENT SERIES

Day Post-Treatment	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin	"t" needed at 0.01 Level of Significance
3	0.52	1.65	-0.16	1.59	0.54	-1.21	
6	-0.08	0.73	-1.43	-0.79	-0.12	-0.12	
9	-0.52	-1.65	-0.33	1.32	0.30	-0.12	
13	0.91	1.38	0.32	1.06	0.48	-0.12	3.07
17	0.77	0.92	-0.33	1.06	0.18	0.54	
21	-1.08	-2.17	-0.82	-1.06	-0.84	1.03	
28	0.39	1.05	-0.33	0.00	0.42	-0.42	
35	0.41	-1.52	-0.33	0.26	-0.90	3.20[x]	
37	-0.50	-0.07	-0.65	0.53	-0.24	-0.72	
39	-0.22	-1.12	0.82	-0.53	-0.84	1.27	
42	1.66	0.00	4.25[x]	0.79	-0.36	2.23	
45	-0.61	0.33	1.63	-0.53	0.24	-2.35	

[x]Significant at P = 0.01.

TABLE 63

STATISTICAL ANALYSIS ("t" VALUES) OF UNINFECTED CONTROL VERSUS 500 + 5,000 TREATMENT SERIES

Day Post-Treatment	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin	"t" needed at 0.01 Level of Significance
9	-1.03	-0.51	-0.95	0.99	-0.67	-0.89	
13	0.25	0.25	0.80	0.00	-0.48	0.44	
17	-0.19	2.99	-0.78	-0.25	-0.85	-1.88	
21	0.47	0.19	-0.64	1.23	0.30	0.44	
28	0.39	1.59	-1.43	0.74	-0.24	-0.66	3.03
35	-0.55	-0.06	0.00	0.99	-1.94	0.50	
37	-2.91	0.25	-2.86	1.23	-2.30	-3.27[x]	
39	0.80	-1.08	1.43	1.23	0.73	1.11	
42	2.61	-0.25	4.61[x]	-0.99	0.24	3.82[x]	
45	-0.89	-1.21	1.43	-0.99	-0.61	-0.44	

[x]Significant at $P = 0.01$.

TABLE 64

STATISTICAL ANALYSIS ("t" VALUES) OF UNINFECTED CONTROL VERSUS 500 + 5,000 + 50,000 TREATMENT SERIES

Day Post-Treatment	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin	"t" needed at 0.01 Level of Significance
17	-0.13	1.13	-0.76	0.00	0.12	-1.25	
21	0.18	0.68	-0.30	0.67	0.88	-1.19	
28	0.00	1.30	-0.61	0.67	-0.35	-0.85	
35	0.98	-0.91	0.91	-0.90	-0.29	3.23[x]	3.03
37	-1.50	-0.51	-1.98	-0.22	-0.99	-1.08	
39	1.21	-1.30	1.67	0.67	0.47	2.72	
42	2.06	0.23	2.89	-1.57	0.41	3.23[x]	
45	0.57	-0.17	1.07	-1.35	0.58	0.85	

[x]Significant at P = 0.01.

TABLE 65

STATISTICAL ANALYSIS ("t" VALUES) OF CHALLENGED UNINFECTED CONTROL VERSUS CHALLENGED VARIOUS TREATMENT SERIES

Day Post-Challenge	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin	"t" needed at 0.01 Level of Significance
control challenged versus 500 challenged							
2	-1.03	0.16	0.00	0.50	-1.14	-1.12	3.03
4	0.39	0.00	0.55	-0.50	0.62	0.11	
7	-0.08	-0.32	0.14	0.75	0.06	-0.11	
10	-1.66	-2.54	0.97	0.50	-0.45	-0.85	
control challenged versus 500 + 5,000 challenged							
2	-1.29	0.37	-0.14	-1.00	-0.74	-2.08	3.03
4	-1.50	-1.00	-1.94	-0.50	-1.31	0.05	
7	-1.00	0.42	-2.22	1.50	-0.40	-1.49	
10	0.00	-1.43	1.39	1.25	0.79	-0.11	
control challenged versus 500 + 5,000 + 50,000 challenged							
2	-0.24	1.27	0.00	0.00	-0.51	-1.33	3.03
4	-1.50	-1.22	-1.53	-1.75	-0.17	-0.64	
7	-0.61	0.26	-1.39	1.50	-0.34	-0.96	
10	-0.39	-2.32	1.39	1.00	1.14	-0.27	

TABLE 66

STATISTICAL ANALYSIS ("t" VALUES) OF UNCHALLENGED VERSUS CHALLENGED SERIES

Day Post-Challenge	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin	"t" needed at 0.01 Level of Significance
			control unchallenged versus control challenged				
2	-1.21	-0.74	-0.83	0.50	-0.62	-0.91	
4	3.79x	0.85	1.80	2.00	1.76	4.05x	2.66
7	3.11x	1.22	4.99x	-1.50	0.68	2.83x	
10	0.21	1.27	0.28	-0.75	-0.68	-0.16	
			500 unchallenged versus 500 challenged				
2	-1.76	-0.53	-0.28	-0.50	-1.53	-1.39	
4	4.40x	1.74	1.66	2.00	3.18x	3.04x	2.66
7	1.45	0.90	1.53	-1.50	1.08	0.75	
10	-0.87	-1.53	-0.14	0.25	-1.36	1.07	

TABLE 66 (continued)

Day Post-Challenge	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin	"t" needed at 0.01 Level of Significance
500 + 5,000 unchallenged versus 500 + 5,000 challenged							
2	0.26	-0.58	1.53	-1.75	0.80	0.16	
4	1.53	0.74	-1.39	0.25	-0.23	3.04[x]	2.66
7	-0.37	1.85	-1.25	1.00	1.59	-2.35	
10	1.05	0.85	0.42	1.50	0.68	0.16	
500 + 5,000 + 50,000 unchallenged versus 500 + 5,000 + 50,000 challenged							
2	0.08	1.00	0.97	0.75	0.17	-1.23	
4	1.05	0.85	-1.25	-0.50	1.14	0.85	2.66
7	0.40	1.27	0.97	1.75	-0.06	-1.17	
10	-0.76	-0.90	0.69	1.75	-0.11	-1.23	

[x]Significant at P = 0.01.

TABLE 67

STATISTICAL ANALYSIS ("t" VALUES) OF UNINFECTED CONTROL VERSUS 5,000 TREATMENT SERIES

Day Post-Treatment	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin	"t" needed at 0.01 Level of Significance
3	0.45	1.18	0.14	0.44	-0.23	-0.41	
6	2.10	1.36	0.56	1.32	1.31	1.01	
9	-0.91	-2.31	0.57	0.66	-0.54	0.74	
13	-1.16	-0.35	-0.14	-0.44	0.08	-1.89	
17	-0.42	-0.24	0.57	0.22	-0.46	-0.54	2.90
21	0.74	1.66	-0.57	-0.22	-0.54	0.47	
28	0.71	-0.12	0.71	-0.44	1.54	0.07	
35	-0.39	0.35	-0.99	0.22	-1.46	0.47	
37	0.36	2.42	-0.85	1.32	-0.54	-1.55	
39	2.52	-0.35	1.34	1.54	2.00	2.90[x]	
42	1.94	0.24	3.54[x]	0.66	1.15	1.28	
45	-0.19	-0.35	1.56	0.22	-1.15	0.20	

[x]Significant at P = 0.01.

TABLE 68

STATISTICAL ANALYSIS ("t" VALUES) OF UNINFECTED CONTROL VERSUS 50,000 TREATMENT SERIES

Day Post-Treatment	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin	"t" needed at 0.01 Level of Significance
3	3.40^x	4.49^x	0.00	1.32	1.84	-0.07	
6	2.72	2.84	0.85	1.32	1.00	0.74	
9	0.45	1.12	-1.42	0.44	0.38	-0.14	
13	-0.16	2.72	-0.57	-1.54	-1.69	-1.22	
17	-0.42	0.89	-0.43	0.22	0.00	-1.76	2.90
21	0.32	1.54	-0.57	0.66	0.15	-1.15	
28	0.84	0.12	0.00	-1.10	1.46	0.68	
35	0.29	-0.24	0.85	-1.76	0.31	0.74	
37	-0.29	0.53	-0.99	0.22	-0.46	-0.41	
39	2.85	-0.83	0.99	1.76	2.68	3.51^x	
42	2.20	0.24	3.12^x	-1.11	1.77	1.62	
45	0.58	0.06	1.28	-0.66	-0.46	1.15	

^{x}Significant at P = 0.01.

TABLE 69 .

STATISTICAL ANALYSIS ("t" VALUES) OF CHALLENGED UNINFECTED CONTROL VERSUS CHALLENGED VARIOUS TREATMENT SERIES

Day Post-Challenge	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin	"t" needed at 0.01 Level of Significance
			control challenged versus 5,000 challenged				
2	0.00	1.46	-0.30	1.02	-2.13	-0.18	
4	-2.27	0.76	-1.77	-1.28	-0.87	-2.90x	2.90
7	-2.46	-0.70	-1.33	-0.77	-0.58	-2.53	
10	-1.10	-3.56x	0.89	0.26	0.97	0.62	
			control challenged versus 50,000 challenged				
2	0.76	2.10	0.15	1.02	0.48	-1.42	
4	-2.88	0.44	-1.92	-1.54	-1.55	-3.02x	2.90
7	-1.70	0.25	-1.03	0.51	-0.97	-2.09	
10	-0.61	-2.41	0.74	0.26	2.04	-0.06	

xSignificant at P = 0.01.

TABLE 70

STATISTICAL ANALYSIS ("t" VALUES) OF UNCHALLENGED VERSUS CHALLENGED SERIES

Day Post-Challenge	Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin	"t" needed at 0.01 Level of Significance
			control unchallenged versus control challenged				
2	-0.64	-0.06	-0.29	-0.78	-0.10	-0.62	2.68
4	5.80x	0.57	2.21	2.33	3.89x	5.00x	
7	4.36x	1.97	4.87x	0.26	2.33	1.60	
10	0.72	2.04	0.59	0.00	-1.94	0.18	
			5,000 unchallenged versus 5,000 challenged				
2	-1.06	-1.21	0.29	-1.29	-1.55	0.62	2.68
4	0.57	1.72	-0.74	-0.78	0.49	0.56	
7	-0.38	1.02	-0.15	1.81	0.29	-2.10	
10	-0.15	-1.15	-0.15	0.00	0.49	0.62	
			50,000 unchallenged versus 50,000 challenged				
2	0.45	1.46	0.88	0.00	0.97	-1.67	2.68
4	-0.42	1.91	-0.74	1.29	-1.07	-1.23	
7	0.08	1.97	0.59	2.07	-0.87	-1.97	
10	-0.57	-0.45	0.00	1.03	0.68	-0.93	

xSignificant at $P = 0.01$.

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BIOGRAPHICAL SKETCH

T. K. Jit Singh Mukkur was born April 1, 1940, in Jullundur, Punjab State, India. He completed a part of his elementary education in Gujranwala (now in West Pakistan). Due to the partition of British India into India and Pakistan, he moved to District Ludhiana, Punjab State (India), where he passed his Matriculation in 1954 from Malwa Khalsa High School (an affiliate of the Punjab University). He received a diploma in medical group (F. Sc.) in 1956 from Government College (an affiliate of the Punjab University), Ludhiana, Punjab State, India. In 1960, he received the degree of Bachelor of Veterinary Science and Animal Husbandry from the Punjab College of Veterinary Science and Animal Husbandry (an affiliate of the Punjab University), Hissar, Haryana State, India. From 1960-1961 he worked as Chief Poultry Inspector, Poultry Development Department, Delhi State. He was awarded a junior fellowship of the Indian Council of Agricultural Research in Bacteriology from 1961 to 1963, by virtue of which he pursued the work for the degree of Master of Veterinary Science (Bacteriology) at the post-graduate college of Animal Sciences (an affiliate of the Agra University), Indian Veterinary Research Institute, Mukteswar-Kumaon, Uttar Pradesh, India, which degree was

awarded to him in 1963. He participated in "the Experiment in International Living" in 1963 in the United States before entering the University of Florida in 1964. He worked as a graduate assistant (teaching and research) in the Departments of Bacteriology and Veterinary Science until the present time and has pursued studies toward the degree of Doctor of Philosophy.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1968

Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:

Chairman